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Vol. 42 June, 1959 Number 6

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JOURNALS NEEDED

The May, June, and September, 1958, issues of this Journal are needed for orders, subscriptions, and late memberships. Send them to the Garrard Press, 510 North Hickory Street, Champaign, Illinois. The Association will pay $0.50 plus postage for each copy.

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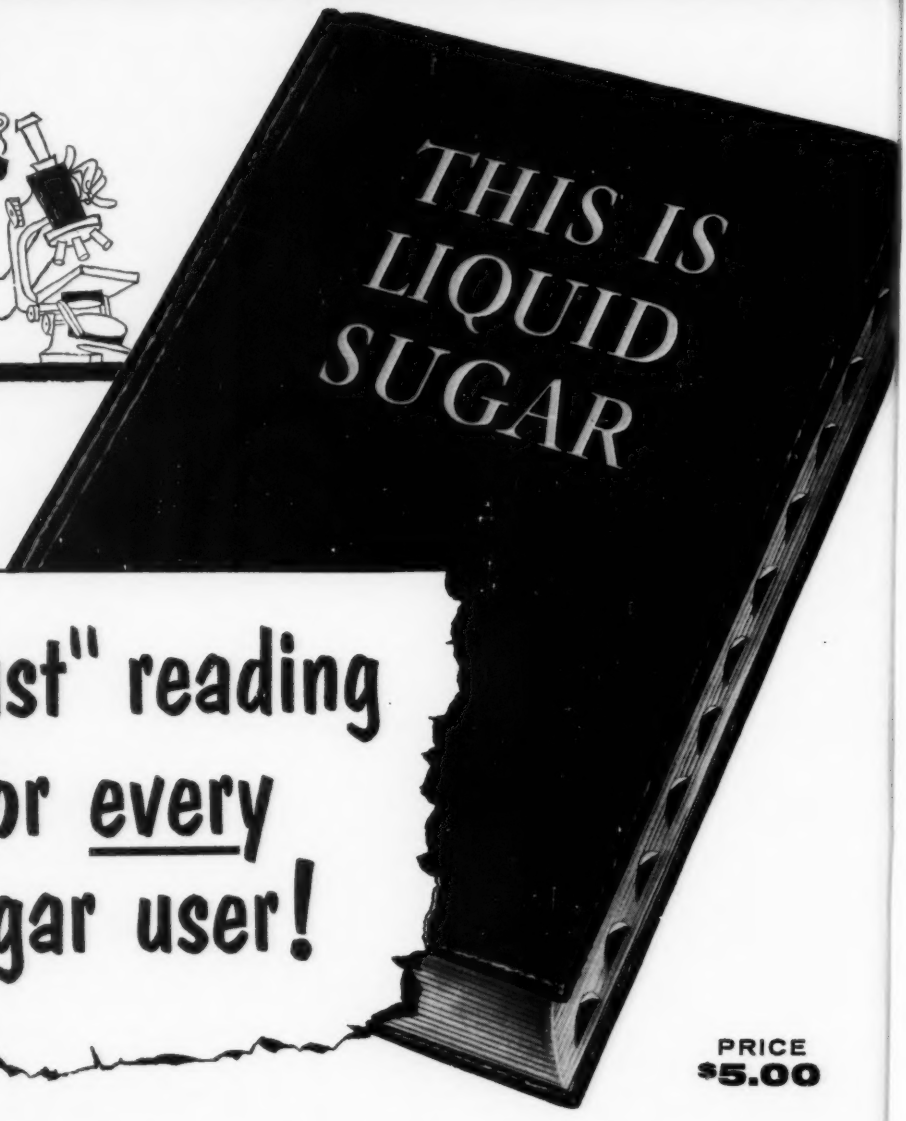

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PEOPLE AND EVENTS

COLLEGE AND UNIVERSITY NEWS

Rhode Island

Dean M. H. CAMPBELL of the University of Rhode Island College of Agriculture retires June 30. Guiding with a friendly, firm hand he has been Dean of the College and Director of the Rhode Island Agricultural Experiment Station since 1942.

Informing his faculty of his decision, Dean Campbell said, "More than 40 years have passed since I entered upon my academic career. I have thought for some time that the completion of those 40 years would be an appropriate time to retire.

"That should have been last year, but I had one ambition which I had not fulfilled. This was to see my faculty furnished facilities in keeping with the outstanding quality of work which it has performed. These facilities are now assured with the completion of the new agricultural building."

M. H. Campbell

F. H. HORN, University president, said, "We are indeed sorry that we shall lose Dean Campbell, for in the 17 years that he has been in charge of the university's agricultural services, notable gains have been made in both academic and research programs . . . he has built a fine organization, strengthened the various departments in the college of agriculture, expanded research programs of the experiment station, and has worked closely with the state agricultural extension service to integrate the station work with the practices of agriculture in Rhode Island.

"We shall have to look hard for a capable successor, but fortunately, we shall have time to fill his position. I know I speak for the trustees and the university family when I wish him only the best in his new career of retirement."

From 1918 to 1927 Dean Campbell was an instructor and associate in dairy husbandry at the University of Illinois, and from 1927 to 1941 professor of dairy production at the University of Vermont. In 1941-42 he was production manager of the Walker-Gordon Laboratories of New England.

Dean Campbell was on leave from the University of Rhode Island between February 1, 1954 and August 31, 1955 to serve as chief agriculturist on a United States Operations Mission in Egypt.

He is a member of the States-relations committee of the experiment station division of the American Association of Land Grant Colleges and State Universities and of the experiment station committee on organization and policy. He is also chairman of the board of the National Project in Agricultural Communications, a trustee of the Eastern States Exposition, a member of the executive committee of the Rhode Island 4-H Foundation, a member of the State Soil Conservation Service and chairman of the scholarship committee of the Rhode Island State Grange.

A native of Genoa, Illinois, Dean Campbell graduated from the University of Illinois with a B.S. degree in 1917. In 1918 he received an M.S. from the University of Wisconsin and in 1932 the Ph.D. degree from the same institution.

California

A new Department of Food Science and Technology has taken over the combined programs in Food Technology and Dairy Industry at the University of California, Davis.

In announcing the change, Dean D. G. ALDRICH, JR., stated that the consolidation of departments was progressive and logical in terms of interest, effort, and expense; and he said that he considered the university fortunate in having a well-qualified scientist to coordinate the new organization.

Heading up the new consolidated department is G. F. STEWART, chairman of the Department of Poultry Husbandry and a biochemist in the Experiment Station.

E. L. Jack

G. F. Stewart

The new head of the Food Science and Technology Department came to California in 1951 from Iowa State College where he was professor of poultry products technology, and chairman of the food technology group.

Dr. Stewart received his B.S. in chemistry in 1930 at the University of Chicago, and his Ph.D. degree in dairy chemistry in 1933 at Cornell University. He was employed in industry as a dairy and poultry technologist before joining the Iowa State College faculty.

As chairman of the Poultry Department at Davis, Dr. Stewart has carried on research in poultry processing. He will continue his work in this field in the new department.

Combining the two food science departments came at a time when E. L. JACK, Chairman of the Department of Dairy Industry since 1946, had requested relief from that assignment to return to teaching and research. He will resume fundamental studies on the chemistry of milk fats, a field in which he has been distinguished since his graduate days at Pennsylvania State University.

Dr. Jack has led a distinguished group of Dairy Scientists on the Davis campus. He and his staff pioneered and successfully implemented a new kind of curriculum in dairy industry. The Department of Dairy Industry has been noted for its fundamental research and outstanding publications.

Dr. Jack is a member of the board of directors of A.D.S.A. and has served on important committees in this organization, including the editorial board of the Journal.

Connecticut

Nine states were represented by men attending the Ultra-High-Temperature Conference sponsored by the Animal Industries Department at the University of Connecticut during April.

E. O. HERREID, University of Illinois, presented a stimulating discussion of the U. H. T. research done there and answered numerous questions. A. M. SHIPLEY, Cherry-Burrell Corporation; H. L. MITTEN, Creamery Package Company; R. C. SODERLUND, De Laval Separator Company, and D. B. HATHERLY of the A. P. V. Company discussed the engineering and operational aspects of the U. H. T. equipment their respective companies manufacture.

In the afternoon a Question-Answer Panel was set up, with the speakers and University of Connecticut Dairy Manufacturing staff members participating. Sanitarians and plant men in the audience provided plenty of questions on this interesting subject.

Florida

P. M. JOYAL, a senior in the College of Agriculture, has received the 1958 Borden Award for the outstanding senior in Agriculture with the highest grade point average. The award was presented by Dean M. A. BROOKER, College of Agriculture, University of Florida.

Mr. Joyal, a transfer student from the University of Vermont, is a major in the Department of Dairy Husbandry. He received his B.S. degree in June. Other awards received by Joyal are the Kroger Scholarship for 1958 and the J. Hillis Miller award for 1958.

He is a past member and officer of the Vermont State 4-H honorary society, was president of the Florida Dairy Science Club, and a mem-

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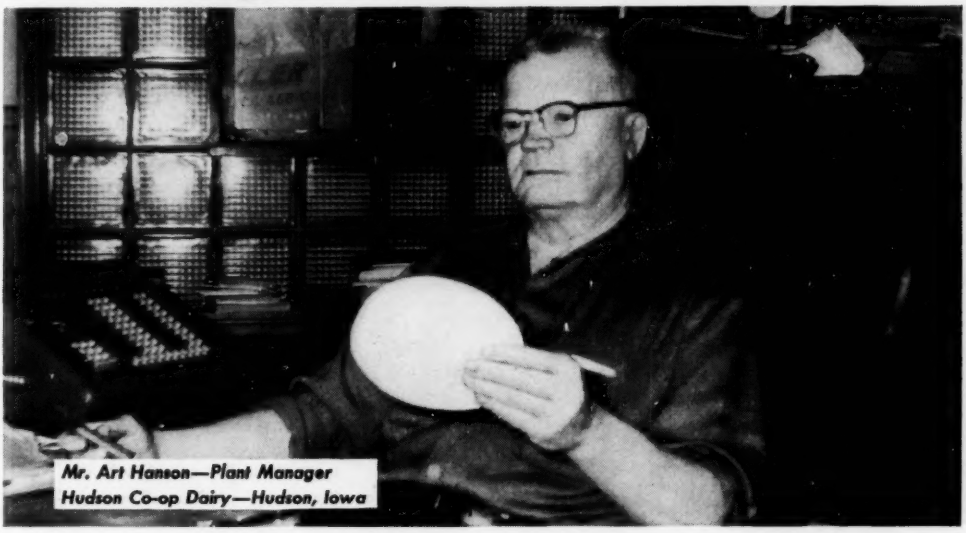

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Dean M. A. Brooker (left) presents the 1958 Borden Award to P. M. Joyal, University of Florida senior. E. L. Fouts, head of the Florida Dairy Science Department, looks on.

ber and Chronicler of Alpha Zeta, honorary agricultural fraternity.

E. A. REGISTER, a senior student in Dairy Manufacturing was presented with the Virginia Dare Award for 1958. The award, which consists of a plaque and a check for $25, was presented for the Virginia Dare Extract Company by E. L. FOUTS, head of the Department of Dairy Science.

Illinois

B. L. LARSON, Assistant Professor of Biological Chemistry in the Department of Dairy Science at the University of Illinois, Urbana, has received a grant of $40,000 from the National Science Foundation to investigate the secretory activity and protein synthesis in mammary gland tissue culture in vitro. Dr. Larson will study factors controlling milk secretion on a cellular basis and of specific milk constituent synthesis. He will work with mammary cells maintained and grown in laboratory culture.

A Short Course in Dairy Technology for southern Illinois dairy plant technicians and other employees has been completed at Southern Illinois University, Carbondale.

Director of the course and principal instructor was R. R. MARSH, associate professor of Dairy Technology, University of Illinois. Other faculty members for the course were JOSEPH TOBIAS, University of Illinois; S. L. TUCKEY, University of Illinois; P. H. TRACY, University of Illinois; and ALEX REED, chairman of the Southern Illinois University Department of Animal Industries.

This non-credit adult education program—which drew some 45 plant operators—was a cooperative venture of the University of Illinois Extension Service and the Southern Illinois University School of Agriculture's Division of Technical and Adult Education.

The Australian Journal Of Dairy Technology

The Journal contains scientific and technical papers on all aspects of dairying, especially dairy manufacture. It provides for those in other countries an up-to-the minute record of the latest developments in dairy technology in Australia and New Zealand. News, reports, editorials, book reviews and Society proceedings are included, and the Journal is illustrated. It appears quarterly.

SOME ARTICLES PUBLISHED IN 1957-58.

"Progress in the Engineering of Dairy Processing" by J. K. Scott.

"Mechanization of Milling, Salting and Hooping in Cheddar Cheese Manufacture" by J. Czulak, N. H. Freeman and H. L. C. Chapman.

"The Stability of Vitamin A in Reconstituted Fortified Non-Fat Milk Solids, Part I—The Effect of Heat; Part II—The Effect of Light"—by J. Conochie and R. A. Wilkinson.

"Modern Packaging of Cheese" by W. J. Park.

"The Effect of Temperature on the Keeping Quality of Butter in Cold Storage" by E. G. Pont and L. F. Gunnis.

"Variations in Lactic Acid Production in Milk" by Barbara P. Keogh.

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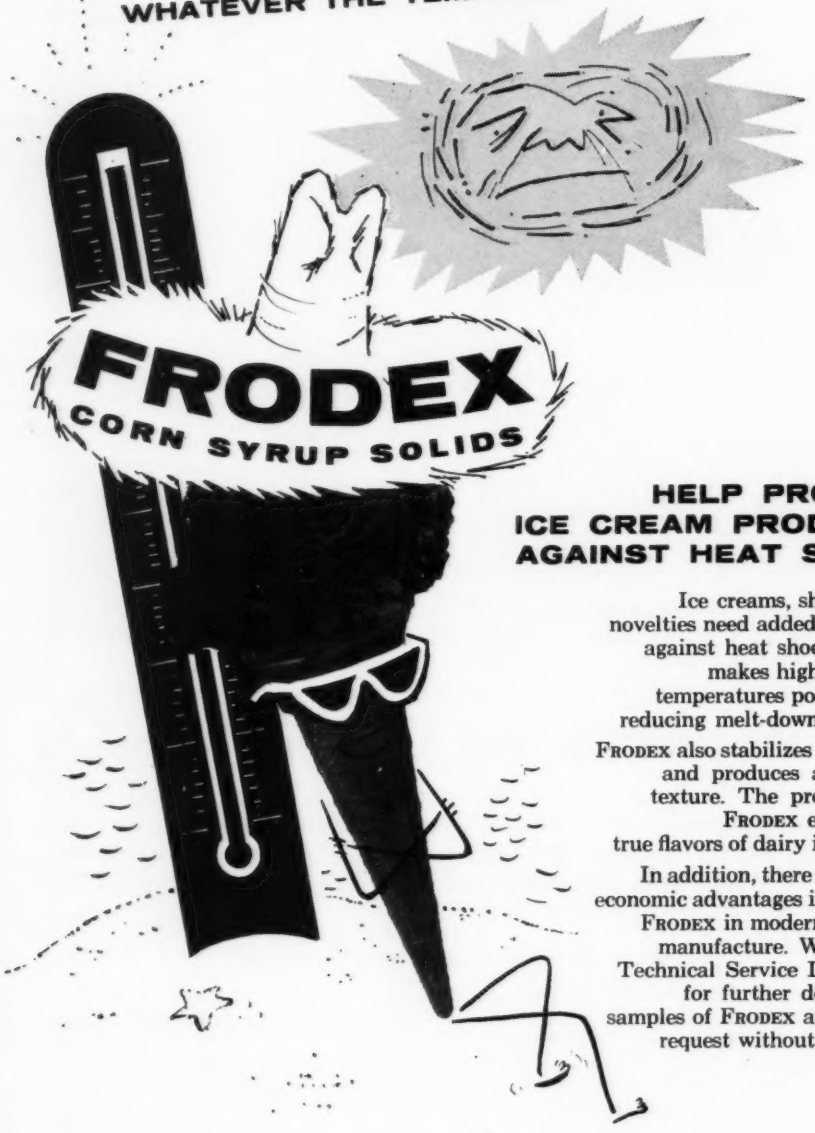

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Nebraska

L. K. CROWE, associate chief adviser of the University of Nebraska Faculty in Turkey reports from Erzurum that spring has heralded the time to get busy with plans to get out into the surrounding area to learn more about the handling of the meager supply of milk and the manufacture of yogurt and cheese.

He reports, "Cows are customarily dry in the winter. We visited a milk cooperative in December which had a barn full of 67 dry cows and some young stock. The cows were all dry and experienced dry periods of from three to five months.

"We are seeing more men—containers of yogurt suspended from both ends of a pole across their shoulders—coming to town from the villages. This indicates that there are more fresh cows."

New Jersey

J. P. MIXNER, Professor of Dairy Husbandry, Rutgers University, New Brunswick, New Jersey, received The New York Farmers Award for 1959 during April.

J. P. Mixner

This prize, which consists of a medal and $500 in cash, is awarded annually to a resident of either Connecticut, Massachusetts, New Jersey or New York for outstanding achievement in the field of agriculture. Mixner's selection was based on his research work in the fields of physiology of reproduction, lactation, and ketosis of dairy cattle with particular emphasis on the roles of the thyroid and the adrenal cortex.

He is to attend the XV International Dairy Congress in London this summer and will present a paper entitled "Thyroxine Secretion Rates in Dairy Cattle as Calculated from Plasma Levels, Turnover Rates and Volumes of Distribution of Thyroxine."

Dr. Mixner is a member of the American Dairy Science Association and is now serving on the Editorial Board of the Journal of Dairy Science.

Ohio

The 6th Annual Dairy Technology Career Day of the Ohio State University brought to the Ohio State University Campus 87 high school students and 37 industry and high school representatives for the purpose of viewing the facilities of the University. They also discussed the curriculum and scholarships in Dairy Technology and the future for graduate Dairy Technologists.

The program consisted of a series of conducted tours through the research, teaching,

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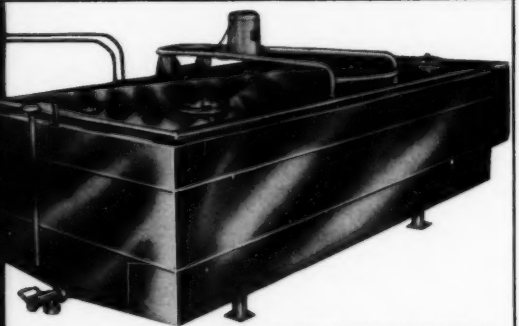

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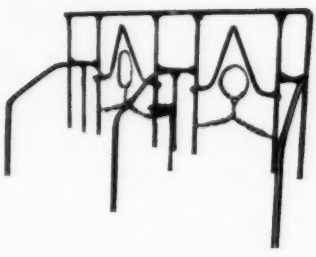

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and operational laboratories of the Department of Dairy Technology; a short formal program which acquainted the visitors with the University, the College of Agriculture and the activities of the Staff and students in Dairy Technology; a luncheon financed by the Dairy Industry of Ohio and served by the Student Dairy Tech Club; and finally group discussion periods which made possible a free interchange of ideas between visitors and the students and staff members of the University.

The Student Dairy Tech Club served as co-hosts for the day and assumed major responsibilities in presenting the story of the Ohio State University and the Dairy Technology Department to the visitors—and serving as members of the panel in the group discussion sessions. A. H. RITCHIE, Assistant Dean of the College of Agriculture, represented the University and I. A. GOULD, Chairman of the Department of Dairy Technology, spoke for the Staff Members in Dairy Technology in the formal program.

In the group discussions, special emphasis was placed on the scholarship program in Dairy Technology which is made possible by contributions from various dairy leaders and organizations of the State. Approximately $11,000 is available this year for scholarship assistance for students interested in Dairy Technology.

As a follow-up to the Career Day, personal interviews and high school scholarship examinations are planned for Toledo, Cleveland, and Columbus. Approximately 45 applicants will be competing for the scholarships.

Texas

R. R. HARRIS has been appointed Adult Education Specialist in Dairying under the general sponsorship of the Texas Education Agency Department of Vocational Agricultural Education.

He will have his office with the Department of Dairy Science on the Texas A & M College campus. His field program consists of conducting short courses for dairymen at locations of high school departments of vocational agriculture. These programs are arranged under the supervision of the Texas A & M Department of Agricultural Education.

J. G. KAPSALIS, formerly of the Texas A & M staff, has been appointed a research assistant at Ohio State University. He will conduct research on the spreadability of butter.

Canada

A conference for Provincial Government Fieldmen was held in April at the Ontario Agricultural College Dairy Department. Sixty fieldmen from all parts of Ontario attended. E. M. BIGGS, Dairy Commissioner for Ontario opened the meeting. R. S. GUTHRIE, Field Veterinarian, New York State, addressed the group on "The function of the milking machine and its relation to the problem of mastitis." O. A. C. staff members presenting papers included F. W. HAMILTON, D. M. IRVINE, and D. R. ARNOTT. Topics discussed at the meetings

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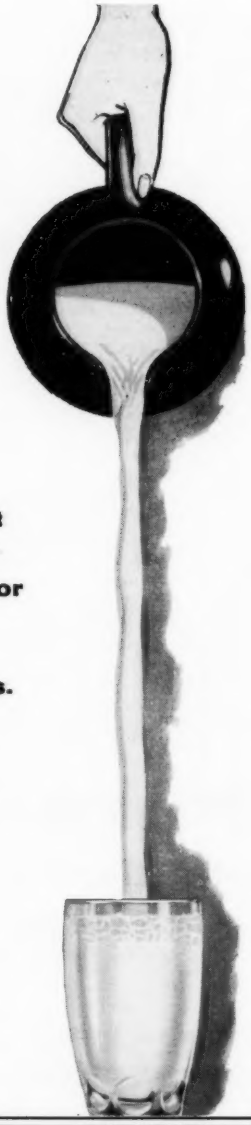

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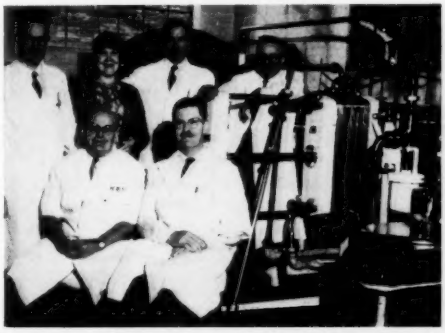
included farm bulk tank sanitation, mastitis control, a milk quality program, and economic trends in the dairy industry.

The Ontario Agricultural College, Guelph, Ontario, has awarded diplomas to 29 students who have completed the three-month Dairy School Course. Five provinces in Canada were represented in the class. This was the 68th Annual Dairy School Course offered at Guelph. The course included cheesemaking, buttermaking, concentrated products, market milk, ice cream, dairy plant sanitation, plant management, dairy chemistry, testing dairy products, and dairy mechanics. Both theoretical and practical aspects were studied. This course has operated annually since 1893 with two courses offered during some of the war years.

The title of the Department of Dairying in the University of Alberta has recently been changed to the Department of Dairy Science. In the past many graduate students have obtained a Masters degree as a result of research work in the department, but up till the present no graduate students in the department had earned the degree of Doctor of Philosophy.



Members of the Alberta Dairy Science Department are (from the left, standing) J. M. de Man, Miss Irene Short, S. Rowe, and F. W. Wood. Seated (from the left) are H. E. Thornton and L. F. L. Clegg, Department Head.

J. M. de Man, who received his training in analytical chemistry, chemical engineering, and dairy science in Holland, has been doing post graduate work in the department for the past four years and has successfully completed all the requirements for a Ph.D. degree and will shortly be recommended for this academic qualification. Mr. de Man's work has been a fundamental study of the physical properties of butter and the practical application of this knowledge to the spreadability of butter, with particular attention to continuously made butter. For this work Mr. de Man has received two special awards for meritorious research from the Alberta Dairymen's Association.

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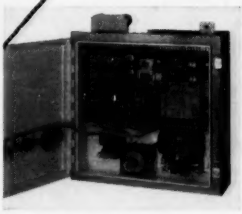

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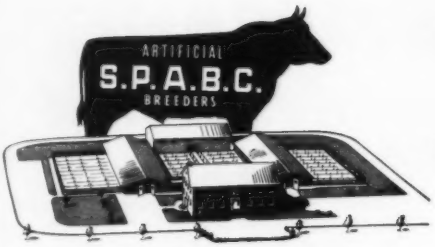

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Much of this work has been done jointly with F. W. Wood under the general direction of H. E. Thornton, the retiring head of the department. It is the intention that this work shall continue.

Mr. de Man will take up duties as Assistant Professor in the Department of Dairy Science as from April 1st. With this appointment there will be a slight re-organization of the teaching in the department. The courses in Dairy Science will be divided into four sections as follows:

1. Dairy Technology under the direction of F. W. Wood; 2. Dairy and Food Bacteriology under the direction of L. F. L. Clegg; 3. Dairy and Food Chemistry under the direction of J. M. de Man, and 4. Dairy Science under the direction of L. F. L. Clegg and staff.

The laboratories of the department are well equipped for research in any of the above four sections. Research in the department receives financial support from such bodies as The Department of Agriculture, Ottawa; The National Research Council of Canada; and the National Defence Research Board. Such grants-in-aid provide for a limited number of assistantships. The assistants if eligible are permitted to register in the Faculty of Graduate Studies and the research findings may be presented to the University as M.Sc. or Ph.D. theses. Various Graduate Fellowships and Scholarships such as those mentioned by the above mentioned organizations are tenable in the department. During the past ten years some thirty scientific papers by members of the department have been published.

The Alberta Dairymen's Association Scholarship in Dairying of the value of $500 is open to undergraduates who have completed the second year in the Faculty of Agriculture and who are registered in a special programme in the Department of Dairy Science. The award is for scholarship attainment during the first two university years with a two-year average of not less than 75%. The Scholarship may be renewed during the fourth year for continued meritorious work.

The Alberta Dairymen's Association also is considering support for students who intend to become dairy specialists and who have completed one summer's practical work in the dairy industry.

Completed Theses

Ph.D. Degree

D. L. Mac Fadden—The relation of the intestinal bacterial virulence as measured by phagocytosis to the mode of action of antibodies in dairy calf growth stimulation. Kansas State University, Manhattan.

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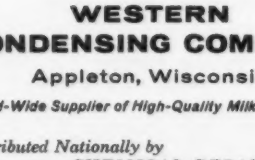

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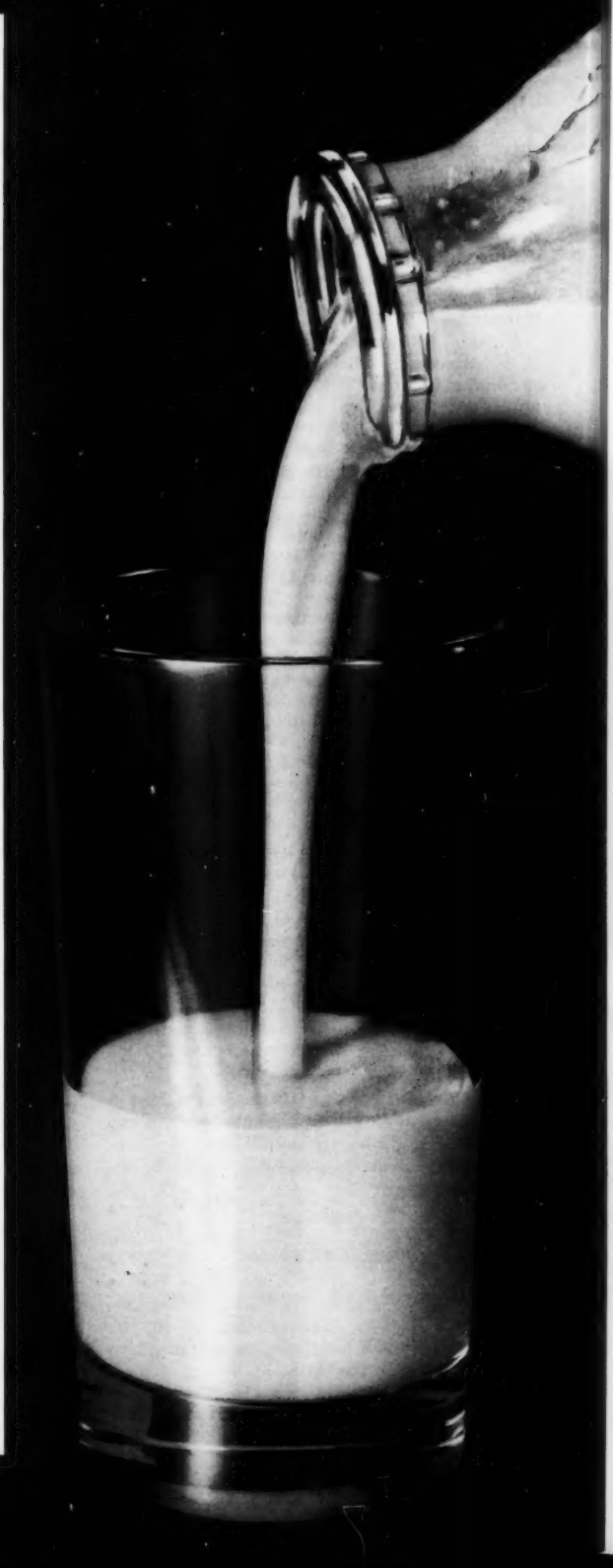


R. R. HARRIS—Effect of supplemental fat in high-energy rations on hot weather performance of producing dairy cows. Texas A & M College, College Station.

J. G. KAPSALIS—The mineral content of water as a variable in the quality control of reconstituted nonfat dry milk products. Texas A & M College, College Station.

C. A. MULAY—The detection of reconstituted milk in natural milk. Texas A & M College, College Station.

M.S. Degree

B. C. PRESNAL—An investigation of the traits of Holstein and Jersey cattle and an empirical test of its implications. Texas A & M College, College Station.

L. E. NAZARENO—The livability of bovine spermatozoa in diluents of various compositions. Texas A & M College, College Station.

B. O. GREGERSEN—A study of the resazurin test on bulk tank milk, Ontario Agricultural College, Guelph, Ontario, Canada.

GOVERNMENT AGENCY NEWS

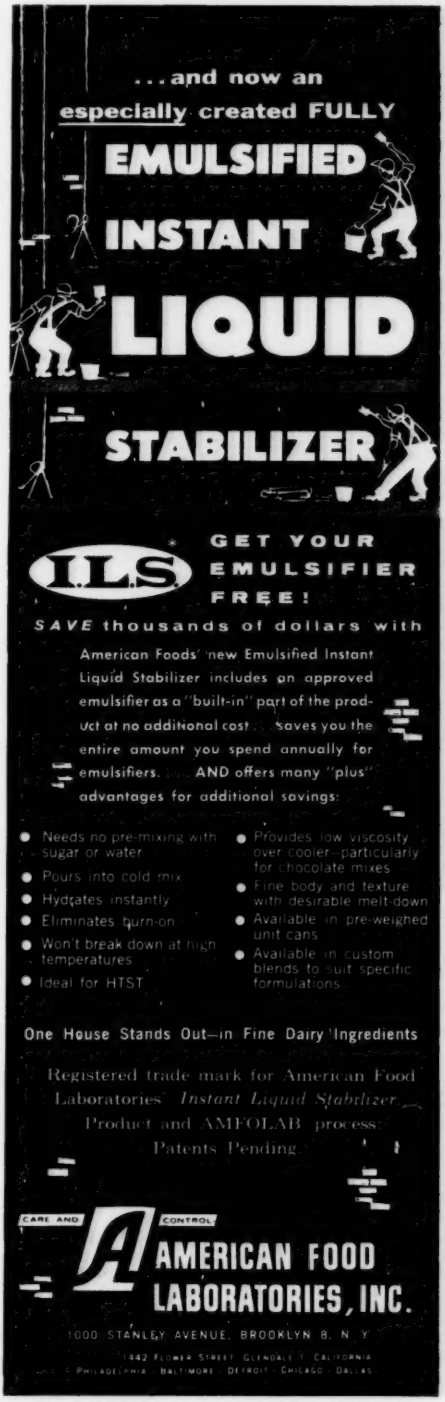
FAO Report on Bombay, India

Plans for a $1,000,000 expansion of the Bombay milk scheme to supply the milk needs of another 1,500,000 people were announced this week by DARA N. KHURODY, Dairy Development Commissioner and Joint Secretary to the Government of Bombay.

Bombay already has the biggest dairy farm in the world, located at the village of Aarey, about 20 miles from the city, where there are 30 modern dairy farms with 21,000 head of cattle. This development, in which the Colombo Plan countries, FAO, and other organizations have assisted the Government, has completely revolutionized the dairy industry in Bombay.

"Bombay is an island city with no surrounding villages from which milk can be sent," explained Dr. Khurody. "The system of milk supply in Bombay was based on keeping the cattle in the city, a very wasteful method as the fodder had to be brought to the cattle and no use was made of the manure. At the same time, the conditions under which milking was carried out, the containers in which milk was transported, and the method of transport were all unhygienic so that milk users always had to boil the milk before they dared use it."

The milkmen and cattle owners lived with their cattle. The traditional pattern was firmly established and, clearly, only the most drastic action by the Government could possibly change the situation. It is just over ten years ago that the Government decided on such action and, as a first step, acquired some 3,250 acres of jungle land at Aarey. A road was built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to the area, water and electricity were installed, accommodation was provided for milkmen and cattle owners and seven dairy farms were built, each accommodating 500 or more cattle.

This part of the plan was completed in 1949 and the Government then asked the city authorities of Bombay to cancel the licenses of milkmen owning 3,500 cattle resident in the city. The owners were then invited to take their cattle to Aarey and work under the new, ideal conditions. While they retained ownership of the cattle, they were required to sell the milk to the Government authority.

"There was at first a great deal of opposition to this scheme, especially to the cancellation of the licences," said Dr. Khurody. "But we were able to resist the pressure to defeat the scheme, including a court case in which opponents to the scheme sought for an injunction.

"But since that time we have steadily progressed so that today there are no cattle at all in the city of Bombay where only a few years ago there were about 21,000," he continued. "All these and more are now located on the 30 dairy farms which we have built at Aarey. I think that everyone concerned in this development is pleased with the result. The cattle are looked after better and kept in good health, and owners and milkmen have better quarters and generally improved social and economic conditions and the consumers have safe, nourishing milk.

"The Government charges the cattle owners a rent of £1 per month per animal," Dr. Khurody explained. "This charge covers not only accommodation for the animal but also for the owner and his milkmen, the Government veterinary services and the artificial insemination service. In actual fact, the money paid by a cattle owner is paid back to him in the price we pay for the milk, which is calculated on the expenses of the owner. In addition, we add a fair percentage to allow the owner a reasonable profit."

The milk, which is produced under hygienic conditions, is pasteurised in a modern plant, bottled, transported to Bombay and distributed. In seven years since the first of the farms started operation the volume of milk handled has grown to 43,000 gallons a day, worth £10,000, a supply sufficient for half the population of Bombay, about 1,500,000. Handling this milk gives employment to 5,000 people.

FAO has helped in developing the Aarey farm colony, having sent four technical assistance experts there and having awarded fellowships to six members of the farm staff to train abroad. FAO also organized at Aarey

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"Progress is the law of life"

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There are many others: "Fresher-tasting" evaporated milk; instant whole milk and instant non-fat milk. And for each product—new or old—Foremost has undertaken a vigorous marketing campaign to educate the public to new uses and new experiences in dairy foods.

Truly, at Foremost progress is the force that vitalizes our business, keeping us abreast of today and ahead of tomorrow.

FOREMOST DAIRIES INCORPORATED

FOREMOST DAIRY FOODS

a training course on tropical dairy farming. It was attended by 44 participants from eleven countries.

"We have now come to the second stage of changing the dairy industry of Bombay," stated Dr. Khurody. "There are still about 30,000 head of cattle in the suburbs and we are now starting on our scheme to eliminate these. To do this we must build another farm colony where we can accommodate some 15,000 of these suburban cattle, allowing economic pressure to eliminate the other 15,000. The milk supply will be supplemented by milk brought from distant villages and the total supply will then be sufficient to meet the needs of the other half of the population of Bombay."

Dr. Khurody has come to Europe to discuss with officials of UNICEF the question of a grant of one million dollars from that Organization to set up the new colony. One of the conditions of the grant will be distribution of $1,500,000 worth of free or subsidized milk to women and children in Bombay during the next five years. At the present time, 75,000 children in the city receive a 6 oz. bottle of milk free each dav.

Dairy Society International Repeats Exhibits in India

In India, an awakened interest in market milk and ice cream, which Dairy Society International sparked through a recombining demonstration at the U. S. Solo Fair in New Delhi was further strengthened at a similar showing in Calcutta which opened March 15 for a month's run. Additional demonstrations are scheduled for Madras in mid-August and Bombay early in 1960.

The Calcutta show is manned by two outstanding dairy leaders of the U. S.—J. S. DETWILER, Chief-of-Mission, export manager of Taylor Instrument Companies, Rochester, New York, and G. H. WILSTER, educator, writer and Professor Emeritus of Dairy and Food Technology at Oregon State University.

With only a two-man team, this exhibit will not follow the usual sampling program used by the Society at the previous dozen or so fairs. Product made during the daily demonstrations of recombining and ice cream making techniques will go to hospitals, orphanages and other designated groups. Only special groups or those interested in the possibilities of enlarging the market milk and ice cream fields will receive samples at the fair at the time of demonstrations especially staged for them.

While this is the first time Mr. Detwiler and Dr. Wilster have represented Dairy Society International in its work, the overseas field is not a new one to either. Detwiler is a well known figure in the industrial world, in food industry planning. He culminated twenty

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Come and see us! Visit our research farm at Gray Summit (above) and laboratory in St. Louis. Like the thousands of dairymen who tour the laboratory and farm each year, we're sure you'll find a visit interesting and worthwhile.

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Dairy Research at MoorMan's

Dairy Research is conducted at MoorMan's by competent scientific personnel working in modern laboratories and in a recently completed Dairy Research Unit, pictured above. Here they carry on controlled experiments with identical twin cows as well as conventional group allotment trials.

Nutrition studies are conducted on cattle of all ages and cover a broad range. These studies include work on such things as trace minerals, hormones, rumen microorganisms, antibiotics, enzymes, vitamins, drugs and palatability factors.

In addition, the effects that different feeding and management practices have on calves and heifers are studied here and in practical field trials. These experiments include levels and systems of feeding . . . their effects on growth, reproduction, general health . . . and on level and persistency of production.

We would be greatly honored to have any or all members of the Dairy Science Association visit with us in Quincy. We would like to show you our modern factory, Research Laboratories and Research Farms. Just drop us a line so we can arrange to have one of our men show you around. We look forward to seeing you and will do our best to make your visit worthwhile. Among the folks from the Research and Service Departments whom you are likely to meet are the following:

W. P. Elmslie, PH.D., Wisconsin—Director of Research
M. J. Caldwell, Ph.D., Kansas State—Head, Nutrition Research
R. A. Sturdy, D.V.M., Ohio State—Head, Veterinary Research
Gerald Brody, Ph.D., Michigan State—Research Parasitologist
Harold Hutchinson, Ph.D., Illinois—Swine Nutritionist
H. C. Eaton, M.S., Illinois—Dairy Nutritionist
A. H. Nesbit, M.S., Illinois—Poultry Nutritionist
Jack Kenney, B.S., Illinois—Dairy Service Representative
A. J. Gehrt, B.S., Illinois—Head, Quality Control
Roger Mittelberg, Supt. of Research Farms and Head, Field Research

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years of technical contributions, through instrumentation to the dairy industry in 1955 when he became head of Taylor Instrument Companies Export Division. His extensive travels have taken him through Europe and Latin America. This, however, is his first trip to the East.

Dr. Wilster, who was born in Denmark, has been an instructor at Australia's Queensland Agricultural College; presented papers at the World Dairy Congresses (1949, 1953); and served as a lecturer in dairy industry at the Royal Agricultural College in Copenhagen, Denmark, under the Fulbright Program. He also has lectured in England, Germany and Australia. Following the Calcutta Fair Dr. Wilster will stay on in India on an approximately six-month assignment with the Food and Agriculture Organization of the U.N., as dairy advisor to the milk colony at Anand.

India has long been a traditional milk-drinking country, but with its teeming millions, all too few have been able to receive milk because of its limited keeping quality, and because the herds of the country, mostly water buffalo, used as beasts of burden as well as dairy animals, cannot supply sufficient amounts. Much has been done through government supervision to improve and properly house the dairy herds, but limited refrigeration and concentration of urban population point to recombining as an answer to a number of India's milk problems.

Actually, the Fair demonstrations are a part of a two-pronged study DSI is making in the Far East. As Cooperator with the Foreign Agricultural Service of the U. S. Department of Agriculture, it has launched a search in India, Burma and Pakistan (and in Brazil in Latin America) for new products combining U. S. surplus milk with local foodstuffs in excess in the host country, to produce a nutritious, inexpensive product which needs little or no refrigeration. For this four-country project, F. E. RICE of the Evaporated Milk Association and FRANK MARTIN, Martin Century Farms, Lansdale, Penna., have been on an extended working tour and are expected back in the U. S. soon.

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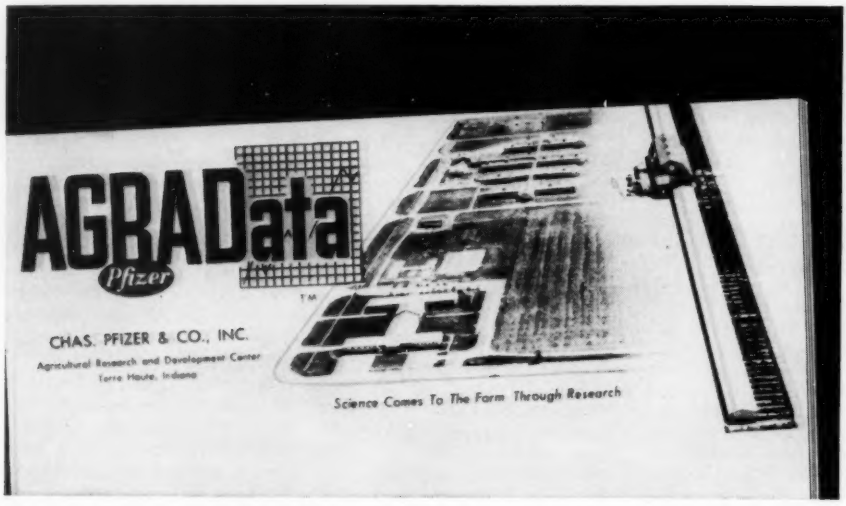
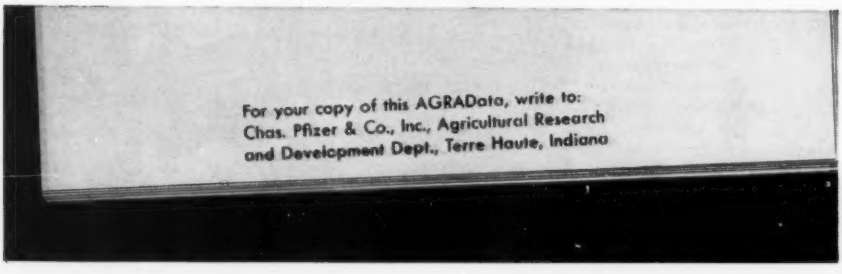


A Review of Research in Breeding Problems

This issue of AGRAData is concerned with research into problems causing $250 million yearly losses to the cattle and dairy industries.

Reports of leading scientists throughout the country who have studied infertility and repeat breeding have been collected into this AGRAData. Recommendations on breeding and overcoming reproductive abnormalities are contained in this issue.

Abstracts of papers presented and published on other phases of livestock nutrition and disease are also discussed. You'll find AGRAData a valuable addition to your library.


For your copy of this AGRAData, write to:
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and Development Dept., Terre Haute, Indiana

INDUSTRY NEWS

Borden's

In a new move to co-ordinate production and sales activitiees, the Borden Foods Company has named three product production managers.

H. C. INGERSON, who has served in various production capacities since joining Borden's in 1945, has been named product production manager of all milk-based products, including evaporated and condensed milk, Starlac, malted milk, Hemo, Dutch chocolate, whole and non-fat powders and related products, and the various can plants.

H. C. STEINKE, formerly associated with the company's cheese operations in Plymouth, Wis., has assumed the duties of product production manager of all perishable products, including package cheese, biscuits, and gelatin salads.

H. L. PARKER, formerly assistant Eastern Division manager located in Syracuse, N. Y., has been named product production manager for the miscellaneous grocery product items: mince meat, instant potatoes, instant coffee, and powdered fruit juices.

The Borden Foods Company also announced that V. R. BUTLER, who has been associated with the company's cheese operations in Plymouth, Wis., since 1932, has been named chief procurement manager for all bulk cheese procurement, storage, and disposal in the Midwest area. In addition, Mr. Butler was appointed co-ordinator of milk procurement for all of Borden Foods' milk plants in Wisconsin. He will continue to make his headquarters in Plymouth.

EDWARD JENSEN, district superintendent of country milk plants in the Orange-Duchess County, N. Y., area, has been appointed supervisor of country plants in the New York Metropolitan Milk District, Borden's Farm Products of New York announced. He succeeds W. R. BAULKWILL, who has retired after nearly 43 years with the company.

In addition to his new duties, Mr. Jensen will continue as supervisor of the Sussex Milk and Cream Company plants in New Jersey.

Mr. Baulkwill, who had been supervisor of country plants since 1935, was also general manager of the company's Reid's Union Dairy Division in Oneida, N. Y., and vice-president of the Hygienic-Northern Division, with operations in Watertown, Adams, Cape Vincent, Evans Mills and Antwerp, N. Y.

Dean Milk Co.

At the Annual Stockholders' Meeting of the Dean Milk Company on April 23, J. M. ROCKWOOD and G. J. HOFFMAN were elected to the Board of Directors, it was announced here today by S. E. DEAN, chairman.

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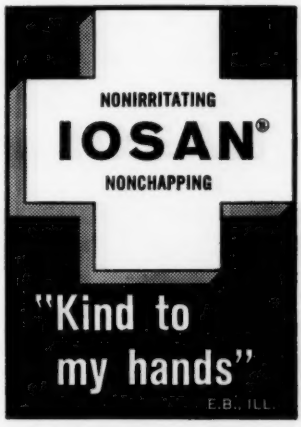

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Mr. Rockwood, vice president in charge of marketing, joined Dean in October 1945. Mr. Hoffman, who joined the company in February of 1947, is president of the Wilson Milk Company, a subsidiary of the Dean Milk Company.

Western Condensing

C. F. MARTIN has been appointed Director of Sales, Western Condensing Company. Mr. Martin succeeds D. L. MANN who has advanced to an executive position with the parent company, Foremost Dairies.

C. F. Martin

In his new capacity Mr. Martin assumes complete sales responsibility for Western's entire line of consumer and commodity products sold to the food, pharmaceutical, and farm industries.

After joining Western in 1955, Mr. Martin was promoted to the position of Sales Manager, Feed Products Division, in 1957. Prior to his association with Western, he worked extensively in sales, including several years with the Minerals and Chemical Corporation of America.

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During World War II he served as a Marine flyer from 1942 to 1946 and was recalled in 1951 to 1954 for the Korean Conflict. He presently holds the rank of major in the Marine Corps Reserve.

Mr. Martin graduated from the National Agricultural College with a Bachelors Degree in Agronomy.

DAIRY TECHNOLOGY SOCIETIES

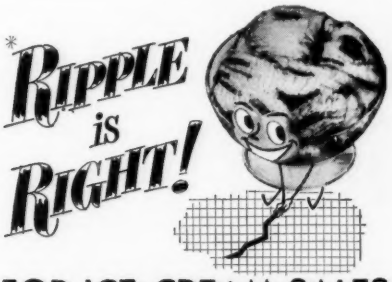
Atlanta—A tour of the Owens-Illinois Glass plant was the featured event of the May meeting. The May meeting was also University night and faculty members from the University of Georgia were honored guests.

Central Illinois—P. H. TRACY, University of Illinois, spoke on the topic "The Impact of Automation on the Dairy Industry." C. V. HOLMES was made an honorary member of the Society.

Central Michigan—D. H. JACOBSEN, director of research for the American Dairy Association, spoke at the May meeting. His title was "Research on Fats and Human Health."

Detroit—"The Status of Concentrated Milks" was the topic of H. E. CALBERT, University of Wisconsin, who spoke at the May meeting.

Kansas City Area—ABRAHAM GELPERIN, M.D., Director of Health and Hospitals, spoke



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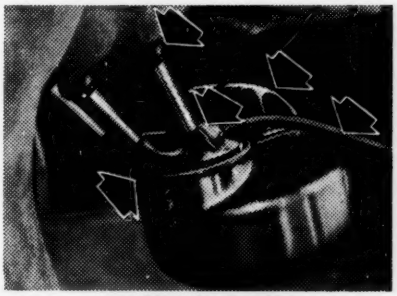

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at the April meeting of the Society. His topic was "What We in the Dairy Industry Can Expect from the Health Department."

Kansas—Ladies Night was the occasion for the May meeting of the Society. The program consisted of the Wichita University Quartet and a dance number from the Wichita dancing school.

Michigan—G. G. RIPMA, Cherry-Burrell Co., spoke on the Topic "Steps in Dairy Plant Equipment and Management." It was the May meeting.

Metropolitan—H. E. CALBERT spoke on the topic "Concentrated Milk" at the May meeting of the Society.

Minnesota—WILLIAM NUNN, Director of University Relations at the University of Minnesota, was the featured speaker at the May meeting. Mr. Nunn's topic was, "Let's Talk About Public Relations."

North Carolina—D. H. LARSON, product sales manager for Cherry-Burrell Corp., spoke at the Society's May meeting. His topic was "Production Planning."

Philadelphia—J. J. FERGUSON, JR., M.D., spoke on the topic "How Cholesterol is Made in the Body." Dr. Ferguson is from Western Reserve University and is working as a Pennsylvania Plan Scholar at the University of Pennsylvania School of Medicine. JOSEPH BONACCI spoke on the topic, "Sodium Fluoride in Milk." J. E. CROSHAW, JR., spoke on the topic "Nervous Problems in the Dairy Cow." The last two speakers are also Pennsylvania Plan Scholars in the School of Dentistry and School of Medicine, respectively.

Tri-Cities—ROLAND BARTLETT, Department of Agricultural Economics, University of Illinois, was the main speaker. His topic was "What Does the Future Have in Store for the Milk Industry?"

Western Michigan—D. H. JACOBSEN, American Dairy Association, spoke on the topic "Fats and Human Health" at the May meeting.

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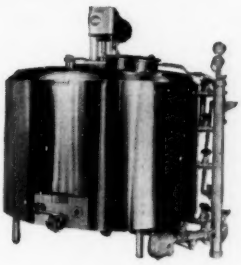

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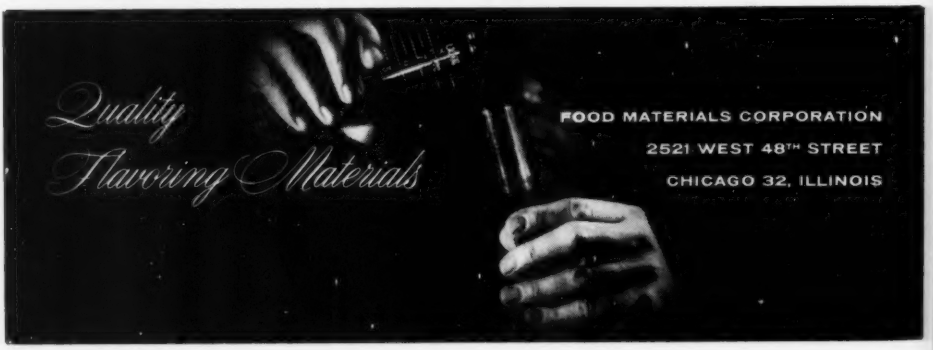

DETECTION OF INTERMEDIATE COMPOUNDS IN THE EARLY STAGES OF BROWNING REACTION IN MILK PRODUCTS[1]

MARK KEENEY AND RICHARD BASSETTE[2]
Dairy Department, University of Maryland, College Park

SUMMARY

A quantitative method is presented for determining 5-hydroxymethylfurfural (HMF) by spectrophotometric measurement if the 2-thiobarbituric acid (TBA) reaction product. A selective digestion procedure, involving the heating of milk with added oxalic acid, is reported for converting early intermediates of the browning reaction to HMF. The method is suggested to be a sensitive detector of early symptoms of the browning reaction. Study of nonfat dry milk (NFDM) indicated that instant products generally exhibited more intensive symptoms of browning than did noninstant products. Spectrophotometry of TBA reaction products is suggested as a promising general quantitative method for the determination of furfurals.

The general significance of the so-called browning reaction in milk products has been discussed in the review by Patton (21). The most important type of browning in these products results from the complex reactions following the interaction of carbonyl and amino compounds. This general type of browning is named the Maillard reaction, after the man who made the first definitive study of the phenomenon (18). Intensive study of the problem in recent years has helped to clarify some of the involved chemical mechanisms and allowed Hodge (10) to present a lucid, integrated scheme of some of the reactions known to play a role in Maillard-type browning. As knowledge has unfolded, it has become apparent that early stages of the reaction, before visible color development, are highly significant and worthy of study, to gain the knowledge necessary to predict and control the browning process. This report presents a sensitive method for detecting early symptoms of browning and its particular application to dry milk.

The reaction scheme in Figure 1 has been established as representative of the early reactions between reducing sugars and amino compounds. This integrated scheme was proposed by Gottschalk and Partridge (8), and substantiated by Hodge and Rist (11) and Richards (25). The scheme is believed to be applicable (10, 25) in explaining the early reactions in natural sugar amine systems. Adachi (1) has identified fractions representative of II and III in evaporated milk and NFDM. Potter and Patton (24) have identified HMF (V) in evaporated milk.

Received for publication June 6, 1958.

[1] Scientific article No. A698, Contribution No. 2921, of the Maryland Agricultural Experiment Station, Dairy Department. Research was undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces and assigned No. 934 in the series of papers approved for publication. The views or conclusions contained in the report are those of the authors and are not to be construed as reflecting the views or endorsements of the Department of Defense.

[2] Present address: Department of Dairy Husbandry, Kansas State College, Manhattan.

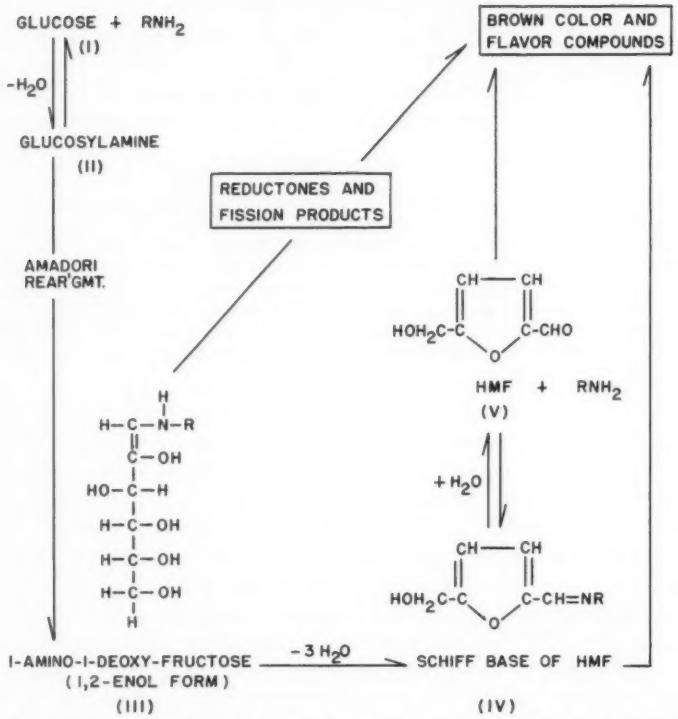

FIG. 1. Scheme of Maillard-type (18) browning reactions involving glucose and amino compound (7, 8, 10, 11, 21, 25). Lactose and galactose will follow the same scheme (1, 22) with hydrolysis of the lactulose disaccharide linkage as an additional step between III and IV.

The proposed method for the detection of browning is based upon analysis for HMF by spectrophotometric measurement of the TBA reaction product. Features which recommend the method for this purpose are the relative stability of HMF and the clear demonstration by Gottschalk (7) that formation of HMF from natural systems, under the proposed digestion conditions, is a specific chemical test for 1-amino-1-deoxy-2-ketoses (III). The method is, consequently, a very sensitive detector of one of the very early reaction products between hexoses and amino compounds.

EXPERIMENTAL PROCEDURE

Identification of HMF in manufactured milks. The observation which led to the present study was that reconstituted NFDM's developed a yellow color when reacted with acidic TBA, and the color intensity seemed to be highest in the stale, flavored samples as compared to those with mild flavor. Further investigation demonstrated that lactose was the component of milk being degraded to the TBA-reactant. This was determined by heating dilute mineral acid solutions

of individual milk components and observing that heated lactose yielded yellow color in the TBA test, whereas casein and ascorbic acid yielded no yellow TBA color. Pure sugar solutions were then heated under standardized conditions and it was observed that fructose yielded more yellow TBA color than did lactose, glucose, or galactose. The preliminary observations suggested that HMF may have been the compound reacting with TBA to produce the yellow color.

Synthetic HMF was prepared by the method of Patton (20) and it was found to react with TBA to produce a yellow pigment with the same spectrophotometric characteristics as the pigment from the milk and sugars. Confirmation that HMF was the reactive agent in the milks was obtained by chromatographic isolation of HMF from the digested stale milks. Ten-milliliter quantities of cooled filtrates from the hot digestion of milk with oxalic acid (Method B, described later in paper) were neutralized by the addition of solid sodium carbonate and extracted three times with 10-ml. portions of ethyl ether. The ether extracts were combined and evaporated to yield a small amount of residue. The residues were spotted on Whatman No. 1 filter paper alongside pure HMF. Chromatograms were developed in two different solvent systems: (a) isoamyl alcohol-formic acid, (b) isoamyl alcohol-ammonia-water, by the method of Potter and Patton (24). The dry chromatograms were sprayed with a mixture of equal volumes of .05 *M* TBA and 40% trichloracetic acid (TCA). A yellow spot congruent with HMF was obtained on both types of chromatograms from the residues of stale NFDM, whereas no detectable spot was obtained from the residues of raw skimmilk. The Rf values for HMF in the two systems were: (a) .74, (b) .63, in close agreement with the values of Potter and Patton. The chromatographic evidence and the spectrophotometric data in Figures 2, 7, and 8, considered in the light of the previous identification of HMF in evaporated milk by Potter and Patton (24), is considered proof that yellow color development in the proposed milk test is due to the reaction of TBA with HMF.

Landucci *et al.* (14, 15) have studied the TBA reactants of commercial proteins and concluded that products of the Maillard reaction were present in the proteins and yielded yellow-red pigments in the TBA reaction. The spectra presented by Landucci (15) of the reaction products of furfural compounds with TBA are similar to those in Figure 2. His reaction conditions involved the heating of proteins or furfurals with TBA in acetic acid solutions and, as might be expected from examination of Figure 4, he obtained about one-third as much color from a given quantity of HMF as could be obtained by using the stronger acid conditions (illustrated in Figure 4). It is recognized that a wide variety of aromatic carbonyl compounds will react with TBA to produce yellow pigments (4). Fat oxidation products (2, 5, 23) may interfere with the test for HMF and browning in certain instances. This type of interference is not serious because it can be easily recognized. Oxidized fats will invariably produce red pigments (23) in addition to possible yellow pigments in the TBA test (5). The presence of enough aromatic carbonyls in a food sample to interfere with the test would usually be detected by general observation and could be confirmed by spectrophotometric and chromatographic techniques.

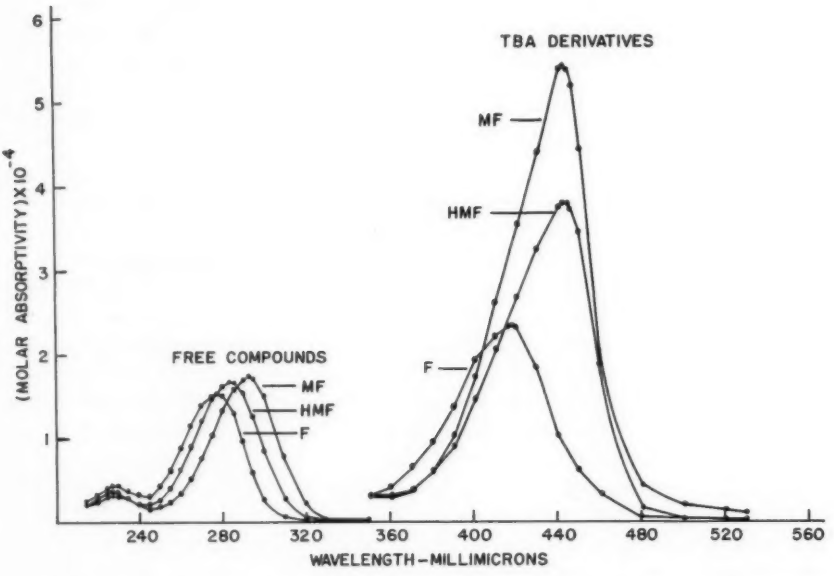

FIG. 2. Absorption spectra of furfural (F), 5-methylfurfural (MF), 5-hydroxymethylfurfural (HMF), and their 2-thiobarbituric acid (TBA) derivatives. Absorptivities of derivatives calculated from original concentration of free furfural compounds by assuming complete reaction between furfural compounds and TBA.

General determination of furfurals by TBA reaction. The authoritative text on furans by Dunlop and Peters (6) discusses the various methods that have been suggested for determination of furfurals and points out the need for further development in this field of analysis. Gravimetric methods, based upon TBA precipitation of furfurals, were presented by Dox and Plaisance (3). The present study is believed to be the first report on the quantitative determination of furfurals by spectrophotometric measurement of the TBA reaction products. While the main objective has been to develop the HMF method for detection of early stages of browning in milk, it was apparent that the experience gained might be applied in the general determination of furfurals. The data in Figures 2 and 3 point out this possibility. The furfural (F) was a freshly distilled Eastman product. The 5-methylfurfural (MF) was a freshly distilled product prepared from sucrose by the method of Rinkes (26). The HMF was a freshly distilled product prepared from sucrose as described by Patton (20). The absorption spectra of the free furfural compounds (Figure 2) were obtained on 1×10^{-5} *M* solutions of the compounds in water. The spectra of the TBA derivatives (Figure 2) were obtained on water solutions containing 16% (w/v) trichloracetic acid (TCA), 1×10^{-2} *M* concentration of TBA, and 1×10^{-5} *M* concentration of furfural compound.

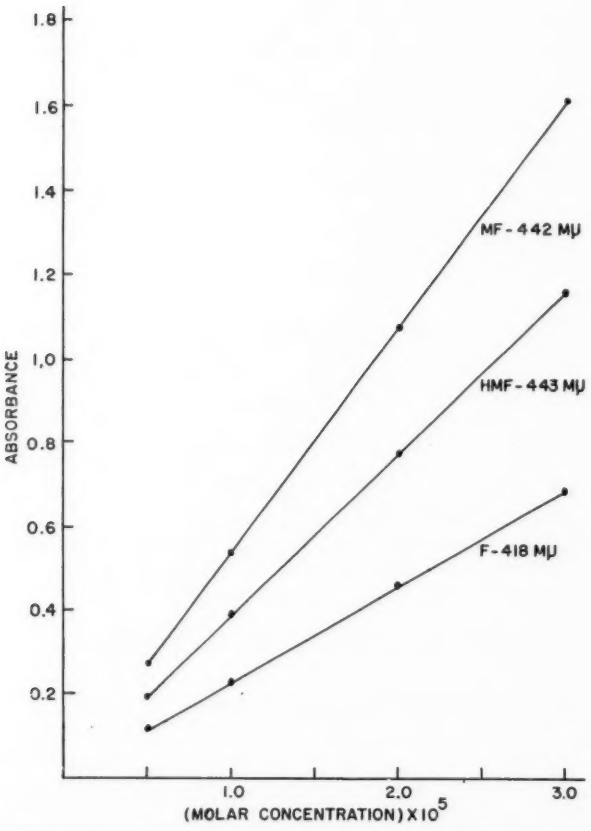

FIG. 3. Relationship between concentration of furfural compounds and intensity of color developed in the TBA reaction.

The derivative solutions were incubated at 40° C. for 50 min., then cooled to 25° C. before determination of spectra. A Beckman DU Spectrophotometer with 1-cm. silica absorption cells was used for this work. Figure 3 illustrates the linear relationship between concentration of furfural compound and the intensity of color developed by the TBA reaction. These derivative solutions are the same as just described for Figure 2, except that the concentrations of furfural compounds were varied from 5×10^{-6} *M* to 3×10^{-5} *M*. The use of a 16% concentration of TCA at this point was somewhat arbitrary, but a detailed study of various reaction conditions between HMF and TBA is presented later in this paper. It has not been possible to isolate the reaction products between furfural compounds and TBA in forms in which they can be redissolved to yield appreciable color. Attempts to purify and recrystallize the reaction products

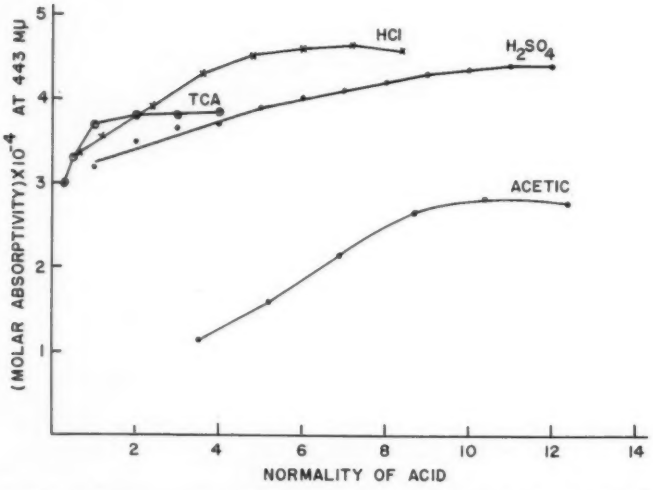

FIG. 4. Effect of concentration of various acids on intensity of color developed in reaction between HMF and TBA. Absorptivities calculated from concentration of HMF. Color developed at 40° C. for 30 min., except in the acetic acid systems, in which it was developed in a boiling water bath for 15 min. HMF concentration 1×10^{-5} *M*; TBA concentration 1×10^{-2} *M*.

resulted in decreases in solubility and light-absorbing properties, apparently due to polymerization.

Study of color-developing reaction conditions between HMF and TBA. The most influential factors in this reaction appear to be heat and hydrogen ion concentration. In the presence of strong acids the application of heat causes degradation of the pigment. Study of various reaction times and temperatures in systems in which the hydrogen ions were contributed by TCA, HCl, or H_2SO_4 indicated that a reaction temperature of 40° C. for 30 to 50 min. was optimum. Higher temperatures caused dimunition of color. However, in systems in which the acidity is contributed by weak acids, such as acetic, higher reaction temperatures are needed for the development of appreciable color, even though the intensity of the color will never be as great as in the strong acid systems. A high concentration of hydrogen ions is necessary for maximal color development. Figure 4 illustrates the effect of various acid concentrations on the intensity of color developed between HMF and TBA.

Digestion conditions for formation of HMF from browning intermediates in milk. The major objective of this work was to develop practical conditions for the formation of as much HMF as possible from browning intermediates (III, IV) without causing primary degradation of lactose to HMF. Figure 5 illustrates the effect of hydrogen ion concentration during a digestion period of 1 hr. in a boiling water bath on the intensity of color developed in the TBA test. It is apparent that pH 3.2 was most selective in distinguishing raw skim from the stale

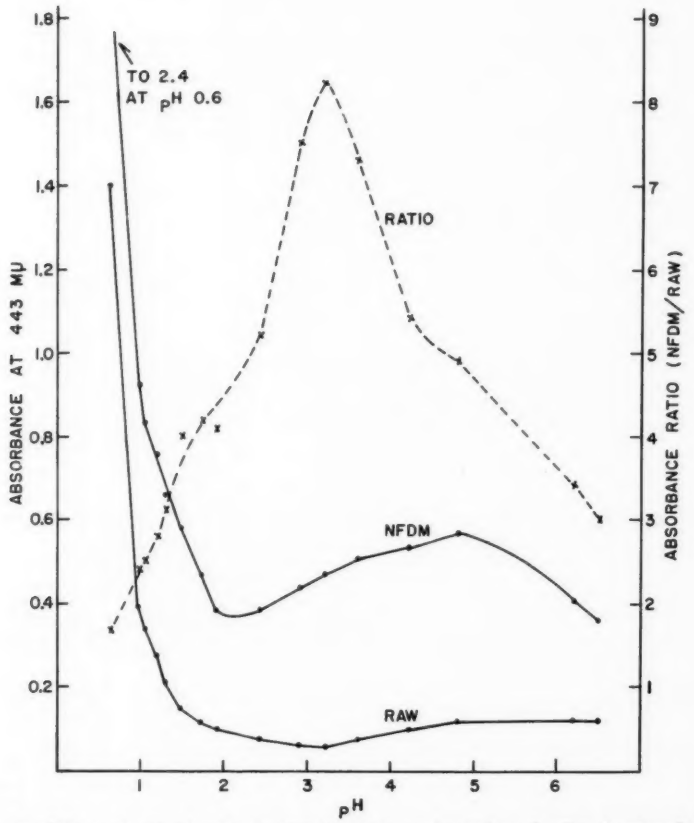

FIG. 5. Effect of pH of milk during digestion on intensity of color developed in TBA test for HMF. Note selectivity of pH 3.2 in distinguishing stale instant NFDM (Xa) from fresh raw milk. Data were obtained by adding 5 ml. of appropriate strength HCl to 10 ml. milk to yield indicated measured pH's, followed by digestion of milk-acid mixtures in boiling water bath for 1 hr., and finishing the analysis by coagulating the cooled samples with 5 ml. TCA (40%) and developing color on filtrates by Method B.

instant milk. The data in Table 1 illustrate the selectivity of oxalic acid and a digestion time of 1 hr. in distinguishing between the two milks. The data in Table 2 point out the necessity of applied heat during digestion for the formation of HMF. These data also show that fresh milk does not yield much HMF during the hot oxalic digestion, whereas the reconstituted dry milks yield relatively large amounts of HMF. Table 2, as well as Figures 7 and 8, indicate that in browned milk products the enol (III) or the Shiffs base (IV) are present in milk in much higher concentrations than free HMF. The concept of detecting III and IV in natural systems, by converting them to HMF with mild acid digestion, was originally suggested by Gottschalk (7).

TABLE 1

Effects of various acids and digestion times on color developed in TBA test[a]

Normality of acid (5 ml.) added to milk (10 ml.)	Digestion time in boiling water bath	Absorbance, 443 mμ		Absorbance ratio NFDM/Raw
		Raw skim	NFDM	
	(min.)			
0.3 H_2SO_4	60	.062	.312	5.0
0.3 H_3PO_4	60	.081	.430	5.3
0.3 Malic	30	.040	.230	5.8
0.6 Malic	30	.038	.230	6.0
0.3 Boric	30	.068	.266	3.9
0.3 Oxalic	30	.031	.218	7.0
0.3 Oxalic[b]	30	.029	.205	7.0
0.3 Oxalic	60	.051	.434	8.5
0.3 Oxalic[b]	60	.063	.438	7.0
0.3 Oxalic	90	.104	.688	6.6
0.3 Oxalic	120	.135	1.02	7.6

[a] Milks analyzed by Method B, except for indicated changes in acids and digestion times. NFDM was freshly reconstituted sample (Xa) of stale, flavored instant NFDM.

[b] Duplicate oxalic experiments conducted on different days with different raw skim; same NFDM.

TABLE 2

Effect of heat during digestion on the intensity of color developed in TBA test (Method B)

Sample	Absorbance, 443 mμ		
	Digestion conditions		
	One hour, boiling water bath (duplicate experiment)		One hour, 25° C. (single experiment)
Noninstant NFDM:			
Xa	.242	.248	.045
Xb	.117	.118	.030
Xc	.156	.156	.032
Instant NFDM from above:			
Xa	.435	.435	.086
Xb	.375	.359	.069
Xc	.428	.421	.066
Raw skim	.056	.057	.028
Fresh pasteurized whole milk	.060	.058	.027

Determination of HMF in milk products. The following alternate Methods A and B are recommended for the determination of HMF in milk products. Elimination of the heating procedure during digestion will result in determination of the free HMF present in the original sample. The application of heat during digestion will convert other browning intermediates (mainly the enol, III) to free HMF. Method A is somewhat arbitrary, compared to B, and is presented only because it was developed early in the work on this project and some valuable data were accumulated with it (12). Method B is preferred because it is based upon a more thorough study and seems to be more selective than A in detecting browning (see Samples Xa, Xb, Xc, Table 3). Figure 6 contains the standard curves from which the formulas were derived for conversion of absorbance values to quantity of HMF. It is pointed out that these methods represent a compromise regarding color-developing conditions with TBA. The use of higher hydrogen ion concentrations would yield more color per unit of HMF; however, the selective

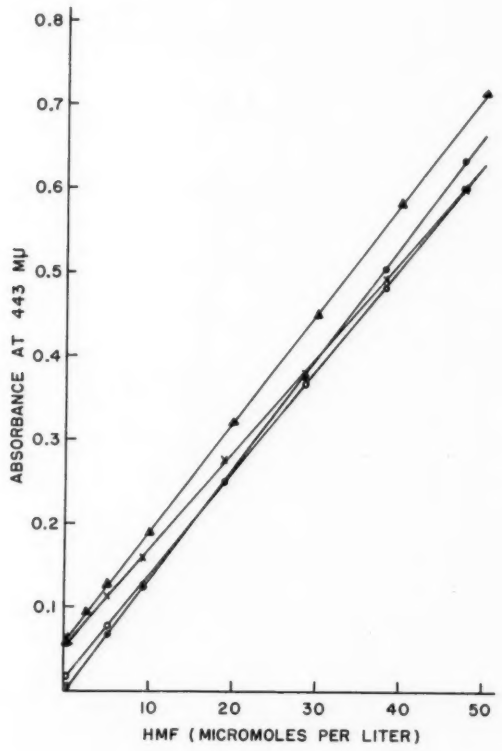

FIG. 6. Intensity of color development as a function of the quantity of synthetic HMF added to milk and water systems in proposed TBA tests. Solid triangles—raw milk plus HMF, Method A. Solid circles—water plus HMF, Method B. Open circles—raw milk plus HMF digested at 25° C., Method B. X's—raw milk plus HMF digested in boiling water, Method B.

quality of the test in distinguishing fresh milk from browned milk would be impaired by the stronger acids causing degradation of lactose in the fresh milk to HMF. A previous report (12) has illustrated the dynamic chemical state of the products of the Maillard reaction in freshly reconstituted dry milk. Therefore, the time of analysis should be standardized and the analysis performed as soon after reconstitution as practical.

Method A: Concentrated products are reconstituted with water to a fluid milk basis before testing and results are expressed in terms of fluid milk equivalent. Nonfat dry milk is reconstituted at the rate of 10 g. of powder to 100 ml. of water. Ten milliliters of milk are pipetted into a 50-ml. test tube. Add 10 ml. of 40% trichloracetic acid solution (40 g. TCA diluted to 100 ml. with water) to the milk from a burette. Mix TCA and milk, cover tube with inverted 20-ml. beaker, and place the tube in a 70° C. bath for 25 min. Remove from bath and

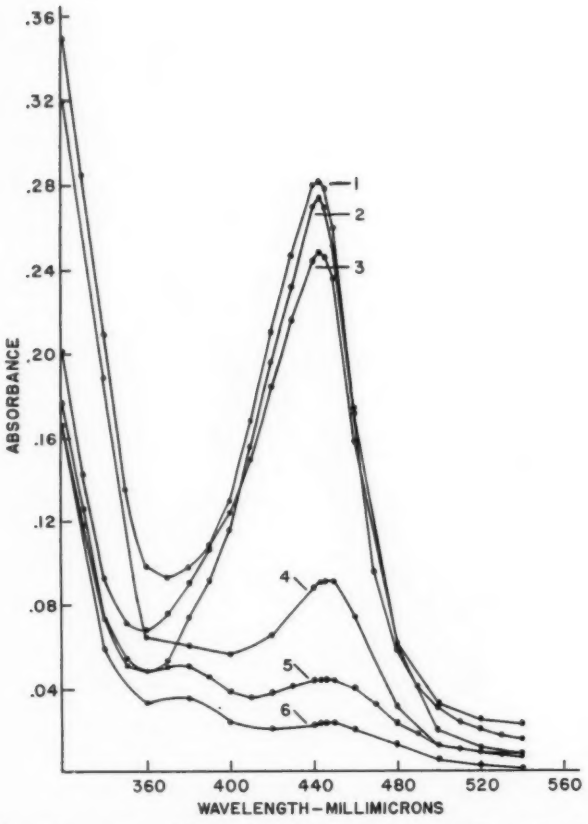

FIG. 7. Absorption spectra of final color solutions of various milks analyzed by Method B. (1) Raw milk containing 19.5 μM per liter of added HMF—digested 1 hr. in boiling water bath with added oxalic acid, (2) same as (1)—digested 1 hr. at 25° C. instead of in boiling water, (3) stale, flavored instant NFDM—digested 1 hr. in boiling water, (4) same as (3)—digested 1 hr. at 25° C., (5) raw milk—digested 1 hr. in boiling water, (6) same as (5)—digested 1 hr. at 25° C.

cool in ice water. Filter contents of tube through Whatman No. 42 filter paper. Pipette 4.0 ml. of the filtrate into a test tube. Add 1.0 ml. of 0.05 *M* TBA (0.72 g. 2-thiobarbituric acid in 100 ml. of water solution prepared by warming slightly and cooling to 25° C. before use). Place this tube in 40° C. bath for 50 min. Remove from bath and cool to room temperature. Measure absorbance of solution at 443 mμ against a blank prepared the same as the sample, substituting water for milk, in a Beckman DU Spectrophotometer or other suitable instrument.

$$(\text{Absorbance} - .062)76 = \text{micromoles HMF per liter of milk}$$

Method B: Prepare sample same as (A) above. Ten milliliters of milk are pipetted into a 50-ml. test tube. Add 5 ml. 0.3 *N* oxalic acid (18.9 g. $H_2C_2O_4 \cdot 2\,H_2O$

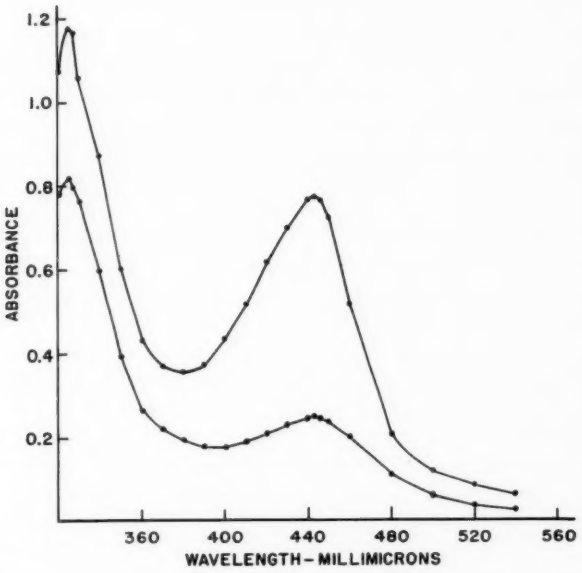

FIG. 8. Absorption spectra of final color solutions of reconstituted evaporated milk analyzed by Method B. Upper curve—digested 1 hr. in boiling water bath; lower curve—digested 1 hr. at 25° C.

diluted to 1 liter with water) to the milk and mix. Cover tube with inverted 20-ml. beaker and place in boiling water bath for 1 hr., after which it is removed and cooled with cold water to room temperature. This heating step is omitted if one wishes to estimate the free HMF in the sample. Add 5.0 ml. 40% TCA, mix, and filter through Whatman No. 42 paper. Pipette 4.0 ml. of the filtrate into a test tube. Add 1.0 ml. of 0.05 *M* TBA. Place this tube in 40° C. bath for 30–40 min. Remove from bath and cool to room temperature. Measure absorbance of solution at 443 mμ again blank prepared same as sample, substituting water for milk, in a Beckman DU Spectrophotometer or other suitable instrument. Calculate HMF from absorbance.

(1) Sample acidified but not heated, which measures free HMF in test milk:
(Absorbance $-.015)81 =$ micromoles HMF per liter of milk

(2) Sample digested 1 hr. in boiling water bath with added oxalic acid, which measures free plus potential HMF from browning intermediates:
(Absorbance $-.055)87.5 =$ micromoles HMF per liter of milk

Application of method to nonfat dry milk. A summary of flavor evaluation and HMF analyses of several brands of NFDM is presented in Table 3. Brands A-K were purchased on the local retail market. Brand X represents nine pairs (before and after instantizing) of NFDM supplied directly from a dry milk manufacturer and analyzed within ten days after instantizing. These data on Brand X give a clear demonstration of the effect of this particular instantizing

TABLE 3

Flavor evaluation and HMF content of nonfat dry milks

Sample, brand[a]	HMF[b] Micromoles/liter		Flavor[c]	
A (I)	5.0 (A)		Slight cereal	(3, 3)
Aa (I)	6.2 (A)		Slight cereal	(3, 2)
A (NI)	4.7 (A)		No criticism	(3, 3)
B (I)	7.4 (A)		Slight cereal	(3, 2)
Ba (I)	7.6 (A)		Cereal	(3, 3)
C (I)	3.9 (A)		No criticism	(3, 3)
C (NI)	4.0 (A)		No criticism	(3, 3)
Ca (NI)	4.6 (A)		No criticism	(3, 3)
D (I)	6.4 (A)		Stale	(3, 3)
Da (I)	5.9 (A)		No criticism	(3, 3)
E (NI)	3.8 (A)		No criticism	(3, 3)
F (I)	6.4 (B)		Cereal	(4, 4)
G (I)	10.7 (B)		Cereal	(4, 4)
H (I)	6.4 (B)		Slight cereal	(4, 4)
J (I)	5.1 (B)		Oxidized	(4, 4)
K (I)	3.9 (B)		Oxidized	(4, 4)
Effect of instantizing:				
Xa (NI)	16.2 (B)	7.3 (A)	Slight cereal	(4, 4)
Xb (NI)	4.9 (B)	3.4 (A)	No criticism	(4, 4)
Xc (NI)	8.1 (B)	5.2 (A)	Slight cereal	(4, 1)
Xd (NI)	7.0 (B)		Slight cereal	(4, 3)
Xe (NI)	8.2 (B)		Slight cereal	(4, 4)
Xf (NI)	6.0 (B)		Cereal	(4, 4)
Xg (NI)	5.0 (B)		Cereal	(4, 4)
Xh (NI)	4.8 (B)		Slight cereal	(4, 4)
Xj (NI)	5.3 (B)		Slight cereal	(4, 4)
Xa (I)	28.3 (B)	15.8 (A)	Slight cereal	(4, 4)
Xb (I)	19.7 (B)	12.2 (A)	Slight cereal	(4, 3)
Xc (I)	23.1 (B)	13.5 (A)	Slight cereal	(4, 4)
Xd (I)	22.3 (B)		Cereal	(4, 4)
Xe (I)	15.9 (B)		Cereal	(4, 4)
Xf (I)	12.5 (B)		Cereal	(4, 4)
Xg (I)	13.2 (B)		Cereal	(4, 4)
Xh (I)	27.8 (B)		Cereal	(4, 4)
Xj (I)	24.2 (B)		Cereal	(4, 4)
Other products:				
Pasteurized whole milk	1.4 (A)	1.5 (B)		
Pasteurized skimmilk	.9 (B)			
Raw skimmilk	.5 (B)			
Evaporated milk	13.3 (A)			
Evaporated skimmilk	35.8 (B)			

[a] Letters I and NI in parentheses indicate instant or noninstant milks, respectively.

[b] Letters indicate method of analysis by Method A or Method B. All samples were analyzed by hot digestion procedures.

[c] First number in parentheses indicates number of judges evaluating sample. Second number indicates number of judges who criticized sample for designated flavor.

process in promoting the browning reaction in dry milk. This confirms the earlier report of Kumetat *et al.* (13), that instant NFDM generally exhibits more intensive symptoms of browning than noninstant NFDM, as measured by acid ferricyanide reducing substances. Caution should be exercised in comparing flavor with HMF values for the samples analyzed by Method A only. These samples (Brands A-E) were analyzed for HMF before discovery (12) of the phenomenon of rapid disappearance of HMF precursors in some samples of freshly reconstituted dry milk. Therefore, the time of analysis, after reconstitution, was not standardized. All of the B analyses were conducted on freshly re-

constituted samples. The general order of increasing flavor intensity of the terms in Table 3 is: no criticism, slight cereal, slight stale, cereal, and stale. Samples J and K, criticized as oxidized, were atypical NFDM's, and this type of flavor, similar to oxidized market milk, appears to be rarely encountered in present-day NFDM. Comparative flavor evaluation of split samples with personnel of the Quartermaster Food and Container Institute demonstrated that the Maryland judges used essentially the same flavor terminology as the QM judges. Flavor terms such as hay and malty were recorded as cereal in compiling the data in Table 3. In general, the data in Table 3 indicate that as the HMF content rises over a critical level of about 5 μM per liter, the appearance of definite cereal-like flavor is more prevalent in NFDM.

Effect of added HMF on properties of nonfat dry milk. The above observations suggested a possible relationship between the intensity of browning, as measured by HMF content of NFDM, and the appearance of cereal flavor. It was also observed that a commercial instantizing process promoted the browning reaction. The following experiment was conducted to gain some insight into the possible direct role of HMF in cereal flavor development. Fresh raw skimmilk was preheated to 150° F. for 30 min., then condensed in a vacuum pan to 29.6% solids. The condensed skim was cooled and stored under refrigeration until the next day, when it was warmed in a hot water bath to 155° F., and spray-dried in a Rogers spray drier. The inlet air temperature to the drier was 265° F. and the exit air temperature was 190–200° F. Forty-pound batches of condensed skim were dried at a time. The drying time for this size batch was about 15 min. The powder was removed from the drier immediately and samples were reconstituted to 8.8% solids and analyzed for flavor and HMF. The first batch dried was the plain condensed skim. The second batch dried was a portion of the same condensed skim to which HMF had been added immediately before drying. The results of the analyses of these milks are presented in Table 4. It is obvious that the added HMF was very reactive during the drying process, as evidenced by the 20% loss of HMF during drying and the concomitant development of strong flavor. This experiment indicates that reaction products of HMF may be involved in the development of off-flavor in nonfat dry milk.

TABLE 4

Effect of spray drying and added HMF on properties of nonfat milk

Sample	HMF[a] $\mu M/l$	Flavor, judge			
		No. 1	No. 2	No. 3	No. 4
(a) Condensed skim	2.4	o.k.	o.k.	o.k.	o.k.
(b) Powder from (a)	4.0	o.k.	o.k.	o.k.	o.k.
(c) Condensed skim + HMF	100.5	Sweet cereal	Flavor o.k., astringent	o.k.	Sweet
(d) Powder from (c)	81.6	Very astringent, old powder, terrible	Astringent, stale, musty, atypical cereal or malt	Malty	Astringent, malty

[a] Method A.

DISCUSSION

A considerable body of evidence from various laboratories has implicated Maillard-type browning as a significant quality determining factor in several manufactured dairy products (9, 13, 16, 19, 21). Early stages of the reaction before visible brown color development are important because associated off-flavors may appear here (21), as well as a possible decrease in the biological value of the proteins (19). Although the major reactants in milk products are probably lactose and proteins (21), Lea (17) has pointed out the possibility that the amino groups of phospholipids may all enter into the reaction. The proposed TBA test for determining HMF is believed to be a promising new objective index of incipient browning in manufactured dairy products, since it is a very sensitive measure of some characteristic early products of the Maillard reaction.

For rough quality control work, the test could be used without a spectrophotometer, because the eye is sensitive to the yellow color and there is a distinct visual difference in color intensity, dependent upon whether the Maillard reaction has been initiated. The major value of the test will probably be its ability to detect the significant initiation of the Maillard reaction, rather than attempted quantitative measurement of the extent or intensity of the reaction. The Maillard reaction is so complex and dynamic that any attempt at stoichiometric measurement of single symptoms should be applied with caution. The purpose of this discussion is not to discourage the quantitative application of the HMF test, but merely to point out its limitations, since the test is a measure of early transitory intermediates in the Maillard reaction. In special cases, and in controlled experiments, quantitative application of the HMF test is valuable, as evidenced by published work (12) and studies of instantizing and drying illustrated in Tables 3 and 4. The HMF test should meet the need suggested by Potter and Patton (24) for a reliable quantitative measure of HMF, in order to study process and storage variables in heated dairy products. It is emphasized again that the procedure of digesting the milks under hot acidic conditions causes the formation of HMF from compounds similar to III and IV (Figure 1), so that in many cases the procedure is mainly measuring HMF precursors or potential HMF rather than actual free HMF. This is particularly true in the case of dry milk. In some dairy products, such as evaporated milk (Figure 8), there may be significant quantities of free HMF in the undigested samples.

Three independent methods of chemical analysis have all indicated that instant NFDM generally exhibits more intensive symptoms of browning than noninstant NFDM. The three methods are: HMF (Table 3); acid ferricyanide reducing substances (13); and indophenol reducing substances (12). It is now clear that some of the commercial instantizing processes accelerate the browning reaction. This is really not surprising, when one considers the general procedures used in instantizing and the information available on the influence of concentration of reactants on reaction rates.

Some of the instant dry milks are manufactured by the wetting of conventional spray-dried milk powder with wet steam to moisture levels of 10 to 20%.

The wet mass is then dried in hot air to approximately 3% moisture, yielding relatively large agglomerated particles of low density, which disperse readily in water. This high-temperature heat treatment of a concentrated milk system, ranging in time from several seconds to several minutes, would certainly be expected to accelerate the browning reaction. Harland *et al.* (9) found a spectacular increase in ferricyanide reducing substances at 10% moisture levels, as compared to higher or lower moisture levels when milk was heated under standardized conditions of time and temperature. Hodge (10) has pointed out that practical attempts to minimize the browning reaction during food dehydration should aim at rapid drying during the final stage of high concentration of reactants. Conventional spray drying meets this objective of rapid drying admirably, while instantizing is a step in the opposite direction. It should be recognized that processes for producing instant NFDM constitute a very valuable technological advance in the dry milk industry, but it should also be recognized that instant NFDM is a new, distinct product having different chemical properties than regular spray-dried NFDM.

CONCLUSIONS

Furfural compounds react with 2-thiobarbituric acid (TBA) to yield yellow pigments which can be quantitatively measured by spectrophotometry. The wave lengths of maximum light adsorption of the TBA derivatives are: furfural, 418 mμ, 5-methylfurfural, 442 mμ, 5-hydroxymethylfurfural, 443 mμ.

A comprehensive study is presented of reaction conditions between HMF and TBA.

A selective digestion procedure, involving the heating of milk with added oxalic acid, is reported for converting early-browning intermediates to HMF.

A quantitative spectrophotometric procedure is presented for determining free HMF and potential HMF in milk products by TBA reaction.

HMF added to condensed milk, before spray drying, is shown to be very reactive during drying to yield off-flavors.

Determination of HMF in NFDM indicates that some of the commercial instantizing processes promote the browning reaction, and the development of cereal-stale type of flavor in NFDM seems to be associated with browning.

The procedure for determining HMF is recommended as a sensitive method for detecting early symptoms of the browning reaction in milk products.

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SOME FACTORS INVOLVED IN THE DEVELOPMENT OF OXIDIZED FLAVOR IN MILK [1]

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SUMMARY

Some factors involved in the development of oxidized flavor were considered. Spontaneous and susceptible milks were used in the study. The milks were subjected to three treatments: raw, pasteurized (63° C. for 30 min.), and heated (76° C. for 30 min.). The factors considered were: the effect of heat, the effect of copper, the effect of hydrogen peroxide, the effect of sulfhydryls, and the role of oxidizing enzymes in oxidized flavor development. The results of the study indicate that an enzyme mechanism is involved in spontaneously oxidized flavor; whereas, a chemical oxidation is involved in induced oxidized flavor. Spontaneously oxidized flavor was prevented either by the use of heat or by an enzyme inhibitor (*p*-chloromercuribenzoate). In contrast, these treatments had no effect upon copper-induced oxidized flavor. The copper-induced flavor was inhibited by the use of a copper chelating agent (2,9-dimethyl-1,10-phenanthroline) and by cysteine. Cysteine was effective in reactivating the enzyme-inhibited milk, thus giving rise to the belief that an enzyme is involved in producing the spontaneously oxidized flavor and that a sulfhydryl group is essential at the active site of the enzyme for enzymatic activity.

There is no marked agreement on identity or role of the factors involved in the development of oxidized flavor in milk. There is agreement, however, that oxidized flavor is the result of the oxidation of unsaturated fatty acids of the milk triglycerides and/or the oxidation of the phospholipids.

Two theories have been proposed as an explanation for the development of the off-flavor; namely, the chemical oxidation theory and the enzyme theory. Kende (11) was the first to report that oxidized flavor was caused by an enzyme system present in milk. The presence or absence of the flavor defect was reported to "be caused by an interaction of metal contamination, oleinase content, and reductases." Since then, several investigators (1, 4–7, 13, 14) have presented evidence to the effect that an enzyme(s) is responsible for the off-flavor. Conversely, other investigators (2, 18) have reported that enzymes are not involved in the development of an oxidized flavor.

Because there is considerable doubt regarding the role of enzymes in the development of oxidized flavor, the present study was undertaken in an effort to determine the cause of this flavor defect in milk.

EXPERIMENTAL PROCEDURE

Materials. A reliable source of milk, which would give rise to spontaneous[2] oxidized flavor, was mandatory for the various studies. Individual samples of

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[2] The classification of milks suggested by Thurston (17) was adopted for this paper. They are defined as follows: Spontaneous milk is that milk which is capable of developing oxidized flavor without copper contamination; susceptible milk is that milk which will develop an oxidized flavor with copper contamination.

milk were taken from 32 cows in the college herd and examined for the flavor defect. Six cows gave milk that oxidized spontaneously. In some instances, milk samples from these cows were used (spontaneous) and, in other instances, mixed-herd milk was used (susceptible). Care was taken to prevent undue exposure of the milk to light and metal contamination. Three different heat treatments were used during the course of the study: (1) control (raw), (2) pasteurized (63° C. for 30 min.), and (3) heated (76° C. for 30 min.). The milks were then subjected to the various treatments as given in the section, Results and Discussion. All samples were stored at approximately 5° C. and were examined periodically for flavor development.

Reagents. The following reagents were used: (a) 1.0 *M* aqueous 2,9-dimethyl-1,10-phenanthroline, (b) 0.3 *N* hydrogen peroxide, (c) 1% cysteine hydrochloride, and (d) 10^{-3} *M* *p*-chloromercuribenzoate.

Methods. The spectrophotometric method proposed by Dunkley and Jennings (9) was used to measure the development of oxidized flavor in milk. Essentially, it consists of measuring with the Beckman DU Spectrophotometer the intensity of color (at 535 mμ) resulting from the reaction between the oxidized flavor components and 2-thiobarbituric acid. In experiments in which oxidized flavor was induced by the addition of ionic copper, the correction factors proposed by Dunkley (8) were deducted from the optical density of the thiobarbituric acid test (referred to hereafter as the T.B.A. test). The results reported are the means of duplicated determinations and are expressed in optical density units.

RESULTS AND DISCUSSION

In anticipation of the use of various substances which might interfere with the organoleptic evaluation of the milk, it was deemed necessary to employ a chemical method for measuring the off-flavor. A comparative study between the T.B.A. test and the organoleptic method showed the T.B.A. test to be a reliable index of the development of oxidized flavor in milk. Furthermore, our data confirmed the findings reported by the workers just mentioned (9). With competent judges performing organoleptic tests, the following correlation was found between the organoleptic and T.B.A. tests: slight oxidized flavor, optical density values greater than 0.055; pronounced oxidized flavor, optical density values greater than 0.070

Effect of heat upon development of oxidized flavor. If an enzyme is responsible for the development of spontaneously oxidized flavor in milk, then the application of high heat should inactivate the enzyme and, thus, prevent the occurrence of the off-flavor. Spontaneous milk was subjected to two different heat treatments. The samples were then stored and checked periodically by the T.B.A. test. The results of two separate experiments are recorded in Table 1. It will be noted that 76° C. for 30 min. prevented the development of oxidized flavor; whereas, 63° C. for 30 min. accentuated its development. The latter observation agrees with that of Kende (11), Chilson (4), and Dahle and Palmer (6). In discussing his results, Dahle concluded that a temperature of 170° F. (77° C.) de-

TABLE 1

Effect of heat treatment upon the development of oxidized flavor in spontaneous milk

Experiment	Treatment	Days at 5° C.					
		0	1	2	3	4	5
		(O. D.)[a]					
1	(Raw) Control	0.027	0.027	0.034	0.035	0.052	0.060
2	(Raw) Control	0.031	0.032	0.034	0.032	0.048	0.056
1	63° C.—30 min.	0.029	0.034	0.036	0.040	0.056	0.065
2	63° C.—30 min.	0.027	0.030	0.034	0.038	0.055	0.065
1	76° C.—30 min.	0.028	0.028	0.035	0.035	0.035	0.035
2	76° C.—30 min.	0.028	0.030	0.036	0.035	0.042	0.042

[a] O.D. = Optical density readings.

stroyed the causative factor; whereas, temperatures of 145° F. (63° C.) for 30 min. and 160° F. (71° C.) for 5 min. did not destroy the causative factor.

From these observations, two explanations for the effect of heat appear possible. One would involve the production of active protein sulfhydryl groups which would act as antioxidants; the other would be the thermal inactivation of an enzyme(s) at 76° C. for 30 min. (the increased flavor intensity at 63° C.—30 min. being merely a reflection of an increase in rate of reaction with an increase in temperature). Data which will be discussed later in this study indicate the involvement of an enzyme(s) and its inactivation by heat at 76° C.—30 min.

Of the oxidizing enzymes normally present in milk, xanthine oxidase (12, 13) and peroxidase (13, 16) are relatively thermostable at normal pasteurization treatments; whereas, catalase (13, 15) is heat-inactivated. Through the sequential action of xanthine oxidase and a peroxidase, it is possible to postulate a series of reactions to explain the development of oxidized flavor. Thus, xanthine oxidase plus substrate yields hydrogen peroxide. The latter in the presence of a peroxidase gives rise to "active" oxygen. Active oxygen plus lipid material could give rise to oxidized flavor.

Effect of copper upon oxidized flavor. In many of the studies dealing with oxidized flavor, ionic copper is added to induce flavor development. A series of experiments was conducted to determine, if possible, the role of copper in induced oxidized flavor.

Three separate lots of susceptible milk were used, because of the time factor involved in determining the T.B.A. values. The copper and copper plus chelating agent were added after the heat treatments. It may be concluded from the data presented in Table 2 that copper-induced oxidized flavor was the end-product of a chemically catalyzed oxidation, because the high heat treatment had no effect in preventing flavor development. This is in contrast to the effect of heat on spontaneous milk (*cf.* Table 1), where the flavor development was prevented by heating to 76° C. It will be seen that the chelating agent (2,9-dimethyl-1,10-phenanthroline) was effective in preventing oxidation both in the raw and in the pasteurized milk. In the heated milk, the chelating agent was effective over a period of three days, but between the third and sixth day oxidized flavor developed to such an extent that the intensity, as measured by the T.B.A. test, was equal to the sample to which 1.0 p.p.m. copper had been added. The significance of the

TABLE 2

Effect of copper upon the development of oxidized flavor in susceptible milk

Treatment	Raw milk				Milk heated to 63° C. for 30 min.				Milk heated to 76° C. for 30 min.			
	Days at 5° C.				Days at 5° C.				Days at 5° C.			
Added copper	0	1	3	6	0	1	3	6	0	1	3	6
(p.p.m.)	*(O. D.)*											
0.0	0.025	0.026	0.026	0.025	0.028	0.032	0.032	0.030	0.026	0.028	0.028	0.033
0.5	0.026	0.026	0.034	0.050	0.032	0.042	0.041	0.056	0.027	0.046	0.066	0.083
1.0	0.028	0.032	0.039	0.058	0.031	0.044	0.048	0.067	0.026	0.051	0.072	0.103
2.0	0.029	0.032	0.048	0.083	0.029	0.054	0.071	0.095	0.032	0.056	0.095	0.161
1.0 + 0.5 Meq.[a] CA[b]	0.026	0.027	0.030	0.060	0.030	0.042	0.046	0.046	0.027	0.034	0.047	0.091
1.0 + 1.0 Meq. CA	0.025	0.026	0.026	0.028	0.031	0.034	0.040	0.044	0.028	0.031	0.034	0.101
1.0 + 2.0 Meq. CA	0.026	0.026	0.026	0.028	0.031	0.035	0.040	0.041	0.026	0.031	0.036	0.101

[a] Meq.=Milliequivalents of chelating agent added with reference to copper content.

[b] CA =Chelating agent, 2,9-dimethyl-1,10-phenanthroline.

latter observation is unknown, unless it is a question of competition between the chelating agent and some coordinating substance present in the heated milk.

In designing the foregoing series of experiments, it was reasoned that one might effectively determine the role of copper in flavor development by the combined use of heat and a copper-chelating agent. The heat treatments, especially the high-heat treatment, should indicate the relationship of an enzymatic oxidation to a chemical oxidation. The controls for the different milks failed to develop an off-flavor; thus, it was concluded that enzymes had little if any effect on the flavor development. In contrast, a comparative study between the samples containing ionic copper and the samples containing chelated copper should give some indication of the role copper plays in flavor development. The results obtained with the heated milk plus copper, although not as definitive as the raw and pasteurized milks, indicated that copper was a catalyst for chemical oxidation.

Effect of peroxides upon oxidized flavor. The oxidation of fats by peroxides is well known. Thus, oxidized flavor in milk partly may be due to the peroxides formed as a result of enzyme activity (e.g., xanthine oxidase) and the oxidation of ascorbic acid. A study was made to determine whether or not the flavor could be induced chemically by the addition of hydrogen peroxide. Spontaneous milk was heated to 76° C. for 30 min. to inactivate the enzymes and then varying amounts of hydrogen peroxide were added to the samples. The results of this study are presented in Table 3. It may be concluded that hydrogen peroxide

TABLE 3

Oxidized flavor development in spontaneous milk as affected by hydrogen peroxide treatment

Teatment	Days at 5° C.			
γ H_2O_2/ml heated milk	0	1	3	6
	(O. D.)			
0 (Raw milk)	0.037	0.042	0.048	0.055
0 (Heated milk)	0.036	0.040	0.036	0.035
15	0.036	0.036	0.040	0.040
25	0.040	0.038	0.040	0.040
35	0.042	0.040	0.035	0.035
50	0.048	0.040	0.040	0.035

when added to milk, in which the enzymes were inactivated, caused no development of oxidized flavor. Thus, it appears that an enzyme is needed for the activation of hydrogen peroxide if this compound is involved in oxidized flavor development. In this connection, it should be noted that hydrogen peroxide, when added to susceptible milk (raw), had a marked effect on oxidized flavor development; thus, 25 γ of hydrogen peroxide per milliliter of milk resulted in a pronounced oxidized flavor, whereas 50 γ of hydrogen peroxide inhibited flavor development. Similar findings have been reported by Cardwell (3). These observations are consistent with the enzyme hypothesis, in that hydrogen peroxide, when present at the lower concentration, serves as a substrate for an enzyme-catalyzed reaction; whereas, at higher concentrations the hydrogen peroxide blocks the enzymatic action.

Effect of cysteine upon oxidized flavor. Several investigators (8, 10, 11, 12, 14, 17) have observed that reducing substances (sulfhydryls) were liberated in milk subjected to high-heat treatment, and they were of the opinion that these substances act as antioxidants to prevent the development of oxidized flavor. Thus, the absence of oxidized flavor in heated (76° C.—30 min.) milk might be a result of the action of sulfhydryl compounds, rather than inactivation of an enzyme(s). To test this possibility, spontaneous raw milk was treated with varying levels of cysteine hydrochloride. It may be concluded from the data presented in Table 4 that cysteine had no inhibitory effect on the flavor develop-

TABLE 4
Oxidized flavor (TBA method) development in spontaneous milk as affected by cysteine hydrochloride

Treatment	Days at 5° C.			
Cysteine · HCl added	0	1	3	6
	(O. D.)			
Control	0.029	0.045	0.057	0.069
10^{-3} *M*	0.031	0.039	0.050	0.065
10^{-2} *M*	0.030	0.040	0.052	0.065
10^{-1} *M*	0.032	0.040	0.050	0.060

ment. The data do not eliminate, but rather support, the involvement of an enzyme(s) in oxidized flavor development. Further, the data give strong indications of the nature of the enzyme involved. For example, cysteine and other sulfhydryl compounds are commonly used as inhibitors for enzymes which contain essential metal ions, e.g., an iron-containing enzyme such as a peroxidase. Therefore, these enzymes apparently are not involved in flavor development. On the other hand, sulfhydryl compounds would have no effect on an enzyme in which a sulfhydryl group is essential for enzymatic activity, e.g., xanthine oxidase. It is recognized that these assumptions must be cautiously interpreted, particularly when only one inhibitor is used; however, it seems logical to assume that spontaneous oxidized flavor is enzyme-catalyzed and that the enzyme involved possesses a sulfhydryl group which is essential for enzymatic activity.

Comparison of enzymatic and chemical mechanisms. It will be recalled that spontaneous milk when heated to 76° C. for 30 min. failed to develop the off-flavor; whereas, the addition of sulfhydryl compounds had little or no effect on flavor development. Likewise, hydrogen peroxide when added to milk heated to 76° C. for 30 min. had no effect. In contrast to this, the copper-induced oxidized flavor occurred independently of heat treatment and was blocked by chelation of the copper. Thus, it appears that at least two distinct mechanisms for flavor development exist; namely, an enzyme-catalyzed reaction for spontaneously oxidized flavor and a chemically catalyzed reaction for induced oxidized flavor. Furthermore, it was suspected that the enzyme involved in spontaneously oxidized flavor possessed a sulfhydryl group which is essential for enzymatic activity.

Experiments were designed to test the validity of these hypotheses. The inhibitor *p*-chloromercuribenzoate was used to determine the role of an enzyme in flavor development, because it is an effective inhibitor for enzymes having an

essential sulfhydryl group. Cysteine was selected to study the role of copper in induced oxidized flavor, because of its ability to bind copper and its ability to reactivate *p*-chloromercuribenzoate-inhibited enzymes. Susceptible raw milk was used in this study and the treatments included all possible combinations of copper, *p*-chloromercuribenzoate, and cysteine with the susceptible milk.

If two mechanisms should be involved in the development of oxidized flavor, then these presumptions could be tested in the following manner. The addition of a specific enzyme inhibitor to susceptible milk (copper-free) should prevent the off-flavor (Sample 2). The addition of cysteine to the copper-free milk should have no effect (Sample 3). The addition of cysteine to enzyme-inactivated milk should give rise to the off-flavor, thus showing that the enzyme involved has an essential sulfhydryl group (Sample 4). The addition of copper to an enzyme-inactivated milk (Sample 6) should give rise to an oxidized flavor, showing that copper catalyzes the oxidation by some pathway other than by enzymes (Sample 6). The addition of both an enzyme inhibitor and the copper-chelating agent to the susceptible milk (containing copper) should prevent flavor development; thereby, showing two different mechanisms of oxidation.

The results of this study are presented in Table 5. It will be seen that there

TABLE 5

A study of the influence of inhibitors upon the development of oxidized flavor

Sample No.	Treatment	Predicted	Found	Days at 5° C.			
				0	1	3	6
				(O. D.)			
1	Raw (control)[a]	+[c]	+	0.028	0.050	0.068	0.074
2	Raw + PCMB[b]	−[c]	−	0.034	0.039	0.043	0.047
3	Raw + Cys	+	+	0.039	0.048	0.067	0.072
4	Raw + Cys + PCMB	+	+	0.031	0.036	0.051	0.065
5	Raw + Cu	+	+	0.037	0.046	0.069	0.075
6	Raw + Cu + PCMB	+	+	0.032	0.038	0.051	0.060
7	Raw + Cu + Cys	+	+	0.040	0.046	0.067	0.067
8	Raw + Cu + Cys + PCMB	−	−	0.030	0.036	0.043	0.048

[a] Spontaneous milk.

[b] Symbols used under sample treatments: PCMB = *p*-chloromercuribenzoate (10^{-3} *M*), Cys = Cysteine hydrochloride (10^{-3} *M*), Cu = Copper (1.0 p.p.m.).

[c] + = Occurrence of oxidized flavor, − = Absence of flavor development.

is good agreement between the observed results and the predictions. Thus, the conclusions are made: (1) That two different mechanisms exist for the development of oxidized flavor (*cf.* Samples 2, 6, and 8); (2) that an enzyme is involved in development of spontaneously oxidized flavor (*cf.* Sample 2); (3) that the enzyme involved in the flavor development possesses an essential sulfhydryl group (*cf.* Samples 2 and 4), and (4) that copper-induced oxidized flavor is a chemical oxidative process (*cf.* Samples 6 and 8).

It is reasonable to assume that the enzyme involved in spontaneous oxidized flavor development is xanthine oxidase, since this is the only known sulfhydryl-containing oxidizing enzyme known to be present in milk. Further work is in progress to study more specifically the role of xanthine oxidase in producing oxidized flavor.

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COMPOSITION OF ULTRAFILTRATES FROM MILK HEATED AT 80 TO 230° F. IN RELATION TO HEAT STABILITY[1]

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SUMMARY

Analyses of ultrafiltrates collected from milk at temperatures up to 230° F. indicated that temperature changes affect the inorganic composition of milk much more drastically than has previously been demonstrated from the analyses of cooled milks. At 200° F., the amount of calcium passing into the ultrafiltrate was approximately 50%, and the phosphate about 82%, of that found at 80° F. On the other hand, the hydrogen ion concentration of ultrafiltrate collected at 200° F. was at least double that of ultrafiltrate collected at 80° F.

Similar tests made with solutions of calcium and phosphate indicated that these changes in the inorganic composition of milk can be explained entirely on the basis of changing solubility and composition of the insoluble calcium phosphate salts. No change in the dissociation of calcium citrate with temperature could be detected.

No correlation was observed between the changes induced by heat and the heat stability of various samples of milk.

Two studies (5, 15) have led to the belief that the inorganic composition of the milk serum is a major factor which controls the heat stability of the milk colloids. However, because the composition of milk sera separated from the proteins at low temperatures has failed to correlate with the heat stability of the colloids (18), it has been suggested that changes in the inorganic composition of the serum during heating control the heat stability (5). No experimental proof for this suggestion has been obtained.

Modification of our recently published (17) ultrafiltration technique made possible the collection of ultrafiltrates from milk over a wide range of temperatures, and a study of the heat-induced changes in serum composition was, therefore, undertaken. Results of this study are presented herewith.

MATERIALS AND METHODS

Mixed-herd milk, and milk from individual cows, was obtained from morning milkings at the Central Experimental Farm, Ottawa. Bulk milk was separated at 90° F. in a cream separator; milk from individual cows was separated by centrifuging for 15 min. at 1,500 r.p.m., in an International No. 2 centrifuge and withdrawing the skimmilk from below the cream layer by siphon. All tests were made on milk which had not been heated before the beginning of the test.

Ultrafiltrates at 80° F. were obtained by the method previously described (17). At temperatures between 80 and 210° F. the apparatus shown in Figure 1 was used. Approximately 600 ml. of milk, preheated to the desired temperature, was poured into the reservoir and a portion pumped into the filter chamber with the needle valve open. Approximately the first 300 ml. of milk flowing from the return line rinsed residual water or milk (several samples can be filtered without

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changing the membrane) from the unit and were discarded. The return line was then attached to the reservoir as shown, and the needle valve slowly closed until the pressure gauge registered 25 lb. From 0.25 to 1 ml. of ultrafiltrate collected in the receiving flask per minute, the amount increasing with increasing temperature. The stainless steel heating coil assured that the milk was within 1° F. of the desired temperature when it entered the ultrafilter. The need for the rotating brush is discussed below.

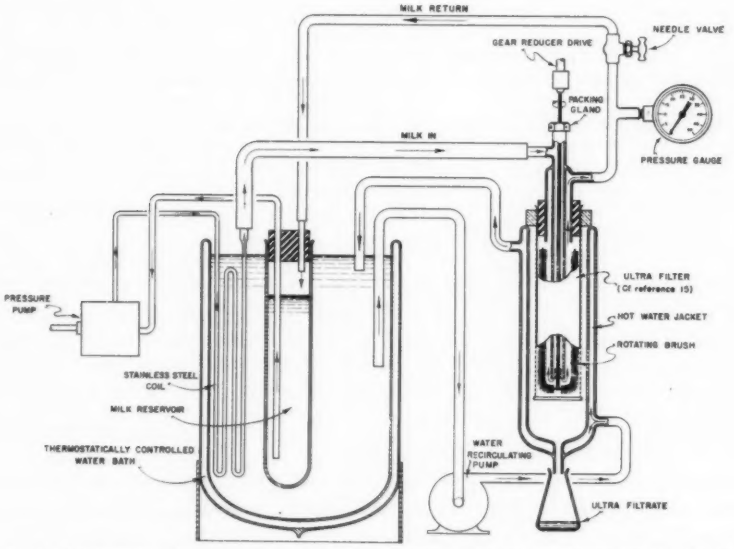

FIG. 1. Apparatus for collecting ultrafiltrate from milk at temperatures up to and including 200° F.

At temperatures above 210° F., the apparatus previously described (17) was filled with hot milk and hermetically sealed into a metal cylinder in which steam could be generated (Figure 2). The pressure relief valve controlled the steam pressure and, hence, the temperature. Air pressure on the milk sample was kept the same as the steam pressure until the milk attained the desired temperature, as indicated by a thermocouple immersed in the milk. Condensate was then drained off through the outlet valve, the air pressure increased by 25 lb., and ultrafiltrate collected in the stainless steel receiver for periodic removal through the outlet valve into an ice-cooled receiving flask.

Ultrafiltrates were analyzed directly by the following procedures: calcium ion (17), total calcium (11), phosphate (14), citrate (12), magnesium (13), and potassium and sodium by flame photometry.

When required for calculation of the product (Ca^{++}) $(HPO_4^{=})$ and $(Ca^{++})^3$ $(PO_4^{\equiv})^2$, concentrations of di- and triphosphate ion were calculated from the dissociation constants derived by Hentola (8) [*cf.* also (17)]. This calculation

ignores the phosphate complexed as undissociated calcium salts, which Bjerrum (2) estimates as between 0.08 and 7%, but the errors thus introduced into the calculated products would not affect the conclusions reached.

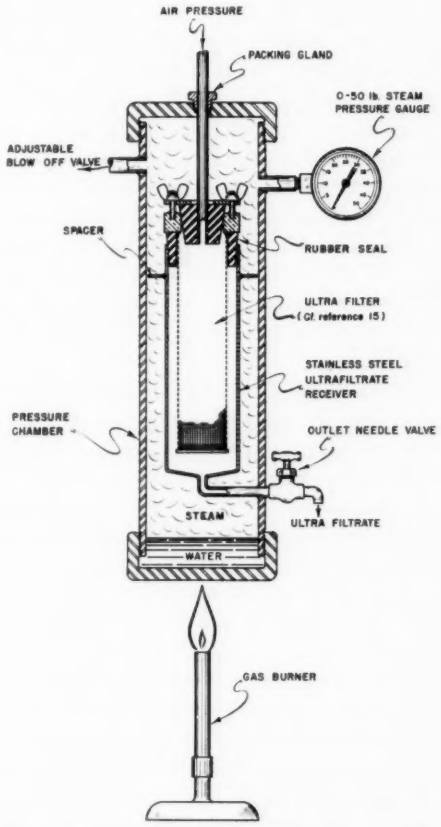

FIG. 2. Apparatus for collecting ultrafiltrate from milk above 210° F.

Heat stability was estimated by sealing 2-ml. aliquots of milk into a 150- by 16-mm. test tube and immersing the tube in a thermostatted oil bath at 284° F. The sample was shaken manually at about 2-sec. intervals, in such a way that milk was splashed on to the sides of the tube. The time required for distinct granulation to show in the milk as it flowed back down the side of the tube was recorded as the "heat stability" of the sample.

RESULTS

Need for the rotating brush. During the early tests with the apparatus, shown in Figure 1, no rotating brush was provided, and a deposit of milk col-

loids formed on the inner surface of the membrane at temperatures above 160° F. Samples of ultrafiltrate collected when this deposit was on the membrane contained abnormally high amounts of several ions, especially potassium, and successive samples collected at one temperature had widely varying compositions. Development of the brushing system shown in Figure 1 made it possible to obtain successive samples of uniform composition, and reduced the potassium content of the serum to that of the original milk.

The interpretation of this phenomenon is of considerable interest but, because of the variability of the results obtained in the presence of the deposit, firm conclusions can not be drawn, except to the effect that brushing of the membrane is essential for reliable results. It appears probable that the serum in the interstices of the deposit becomes more concentrated because ions are attracted to the deposited proteins, and that this concentrated serum is forced outward through the membrane. As the thickness of the deposit increases, the composition of the ultrafiltrate presumably approaches that of the ionic atmosphere around the protein particles in the deposit; whereas, in the absence of the deposit the ultrafiltrate has the composition of the serum itself.

The brushing system was not used with the apparatus for collecting ultrafiltrate from milk above 210° F. and some error thus may have been introduced. However, little deposit of protein formed on the membrane at 230° F. and it is, therefore, unlikely that this error would be large.

Effect of time and temperature of heating. The composition of ultrafiltrate collected at various times from a sample of skimmilk held at 200° F. is shown in Figure 3. The 0 time samples were collected at room temperature before heating the milk. Collection of hot ultrafiltrate was begun 11 min. after the milk had been heated, and sufficient sample for analysis was collected in a further 15 min. This 11- to 26-min. sample had the same composition, within reasonable limits of error, as the later samples, although the pH was slightly higher. Sufficient sample for a complete analysis could not be collected within a shorter interval, but analyses for calcium and phosphate made on smaller amounts of ultrafiltrate collected at short intervals indicated that equilibrium was established within 5 min. at 180° F.

Increasing temperatures progressively increased the loss of calcium ion, total calcium, and phosphate, and decreased the pH of ultrafiltrates from skimmilk (Figure 4A). There was no significant change in the citrate, magnesium, sodium, or potassium content of the ultrafiltrate at any temperature studied. The loss of phosphate and the decrease in pH appeared to be greater per unit temperature change at the higher temperatures; the loss of calcium ion and total calcium was relatively uniform throughout the temperature range studied.

The composition of ultrafiltrates from 2:1 concentrated skimmilk changed with temperature of filtration in the same manner as the composition of those from skimmilk (Figure 4B). The change in pH and calcium ion concentration was of the same order in concentrated and normal samples, but approximately twice as much total calcium and phosphate became nonultrafilterable.

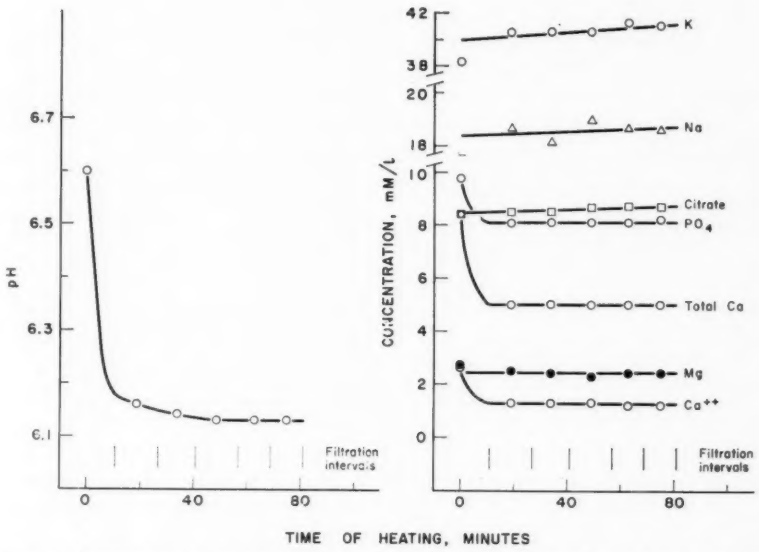

FIG. 3. Composition of ultrafiltrates collected from raw skimmilk and from milk heated to 200° F.

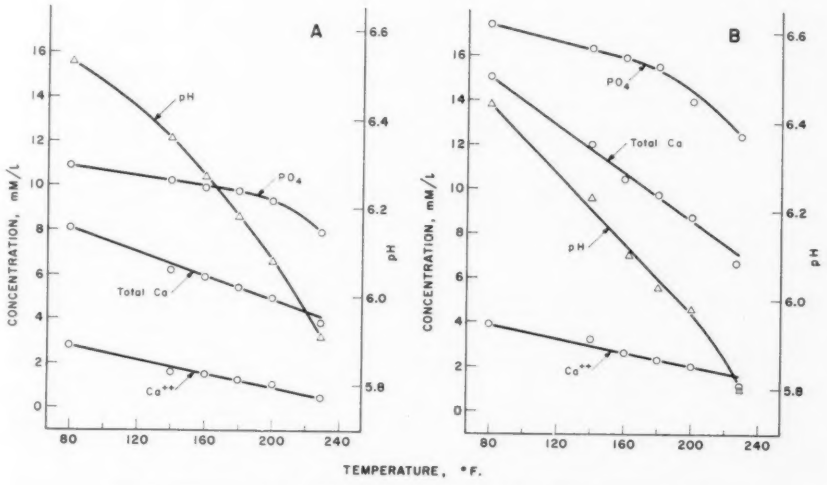

FIG. 4. Composition of ultrafiltrates collected at various temperatures.

A—skimmilk

B—2: 1 concentrated skimmilk (16.3% T.S.)

TABLE 1
Composition of ultrafiltrates from unheated, heated, and cooled skimmilk

Treatment	pH	Composition of ultrafiltrate				
		Ca^{++}	Total PO_4 Ca		$[Ca^{++}] \times [HPO_4^{=}]$	$[Ca^{++}]^3 \times [PO_4^{\equiv}]^2$
		(mM/liter)				
Sample I						
Unheated	6.77	2.6	8.7	8.6	11.1×10^{-6}	6.1×10^{-23}
140° F. 130 min.	6.42	1.9	7.4	8.0	4.7×10^{-6}	0.2×10^{-23}
Cooled 1.5 hr.	6.63	2.2	8.2	8.1	7.4×10^{-6}	1.2×10^{-23}
Sample II						
Unheated	6.83	2.9	8.9	10.2	15.7×10^{-6}	18.4×10^{-23}
200° F. 20 min.	6.22	1.1	5.2	7.6	1.8×10^{-6}	0.6×10^{-23}
Cooled 1 hr.	6.64	2.2	8.4	9.0	8.4×10^{-6}	1.6×10^{-23}
Cooled 4 hr.	6.69	2.4	8.7	9.4	10.2×10^{-6}	3.3×10^{-23}
Cooled 22 hr.	6.73	2.7	9.2	9.8	12.6×10^{-6}	6.7×10^{-23}

When heated milk was recooled, the composition of the serum tended to return to that of the original milk, and the composition of ultrafiltrates collected after cooling for 1 hr. or longer (Table 1) agreed with values for heated milk reported by other workers (8, 10). However, the values for $(Ca^{++})(HPO_4^{=})$ and $(Ca^{++})^3 (PO_4^{\equiv})^2$ remained slightly below those of the original milk, even after 22 hr. at 40° F.

Studies with artificial sera. Heating synthetic sera prepared as in (16), which contained suspended calcium phosphate, to 200° F. altered the composition of the ultrafiltrate in much the same way that heating skimmilk altered its ultrafiltrate (Table 2). However, the drop in pH was more marked in the synthetic sera, and the loss of calcium and phosphate appears to have been somewhat greater.

To determine the effect of heating on solutions of calcium phosphate alone, three solutions of varying Ca : P ratio were prepared at ionic strength 0.08

TABLE 2
Composition of ultrafiltrates from synthetic sera at 200° F.

Heating time *(min.)*	pH	Composition					
		Ca^{++}	Total Ca	PO_4^{---}	Na^{+}	K^{+}	Citrate
		(mM/liter)					
Sample I							
Unheated	6.55	2.1	8.3	9.7	22	37	7.3
5 to 16	5.68	1.1	4.2	6.6	23	38	....
16 to 34	5.64	0.7	3.8	6.8	23	38	7.3
34 to 52	5.60	0.7	3.4	6.5	24	38	7.3
52 to 70	5.57	0.6	3.5	6.5	23	38	7.3
Sample II							
Unheated	6.68	1.8	7.5	7.7	17	28	7.2
5 to 24	5.66	1.0	4.2	5.7	18	32	7.2
50 to 64	5.54	0.8	3.6	5.4	19	33	7.1
90 to 114	5.62	0.6	3.4	4.9	17	31	6.9

(adjusted with potassium chloride), and adjusted to approximately pH 6.7 to induce precipitation. Aliquots of these solutions were then heated for 20 to 30 min. and the precipitate removed in a heated centrifuge. The supernatants were cooled and analyzed. No correction for evaporation of the hot solutions was made, as the increase in concentration (about 10% loss of volume at 200° F.) would merely cause some additional precipitation and not significantly affect either the pH or the composition of the saturated supernatants.

The results (Table 3) indicate that even under these conditions heating the suspensions caused changes to occur in the pH of the supernatants, and in the $(Ca^{++})(HPO_4^{=})$ and $(Ca^{++})^3(PO_4^{\equiv})^2$ products. Precipitation of calcium phos-

TABLE 3

Composition of supernatants from solutions of calcium phosphate centrifuged at various temperatures

(All solutions contained solid calcium phosphate in suspension)

Solution No.	80°	120°	140°	160°	180°	200°
			pH of supernatant			
1[a]	5.98	5.76	5.53	5.19	5.19	4.87
2[a]	6.13	5.83	5.60	5.32	5.22	5.08
3[a]	6.36	6.24	6.19	6.13	6.12	6.07
			Calcium content of supernatant *(mM/liter)*			
1	4.32	4.13	4.26	4.79	4.86	4.93
2	3.22	2.80	2.68	2.66	2.87	2.96
3	0.79	0.40	0.32	0.17	0.16	[b]
			Phosphate content of supernatant *(mM/liter)*			
1	4.50	4.32	4.36	4.70	4.84	5.18
2	6.22	5.94	5.96	6.26	6.40	6.48
3	12.20	12.00	12.31	12.82	12.82	13.56
			Loss of calcium from supernatant *(mM/liter)*			
1	2.58	2.77	2.64	2.11	2.04	1.97
2	2.98	3.40	3.52	3.54	3.33	3.24
3	3.21	3.60	3.68	3.83	3.84	.....
			Loss of phosphate from supernatant *(mM/liter)*			
1	1.50	1.68	1.64	1.30	1.16	0.82
2	1.78	2.06	2.04	1.74	1.60	1.52
3	2.80	3.00	2.69	2.18	2.18	1.44
			Ration of calcium loss/phosphate loss *(mole basis)*			
1	1.72	1.65	1.61	1.62	1.76	2.40
2	1.67	1.65	1.73	2.03	2.08	2.13
3	1.15	1.20	1.37	1.76	1.76	.....
			$[Ca^{++}][HPO_4^{=}] \times 10^6$			
1	2.7	1.6	1.0	0.6	0.6	0.3
2	3.7	1.7	1.0	0.6	0.5	0.4
3	2.7	1.1	0.8	0.4	0.4	....
			$[Ca^{++}]^3[PO_4^{\equiv}]^2 \times 10^{27}$			
1	156	19	2.9	0.22	0.25	0.015
2	440	20	2.3	0.20	0.11	0.034
3	163	78	2.8	0.28	0.21	.....

[a] Before adjustment to pH 6.70, which initiated precipitation, Solution 1 contained 6.90 m*M*/liter calcium and 6.00 m*M*/liter phosphate, Solution 2, 6.20 m*M* and 8.00 m*M*, and Solution 3, 4.00 m*M* and 15.00 m*M*, respectively.

[b] Too low to determine.

phate in excess of that precipitated at 80° F. was not extensive, and occurred only at 120 and 140° F. The further decrease in pH and in the apparent solubility products at higher temperatures was largely caused by a change in the composition of the precipitate (re-solution of phosphate in excess of calcium).

Since temperature could affect the dissociation of any weakly dissociated acids present in ultrafiltrate, a few pH measurements were made at 180° F., with a Beckman Model G instrument and the recommended Beckman procedure. Samples in which a precipitate formed on heating (milk, ultrafiltrate collected at room temperature, synthetic serum) decreased in pH by 0.25 to 0.4 units. Samples in which no precipitate formed, i.e., ultrafiltrate collected at 180° F., supernatant from synthetic serum centrifuged at 180° F., and calcium-free synthetic serum, increased in pH by 0.07 to 0.16 units (average increase, 0.11 unit).

An attempt was also made to utilize the murexide method for the determination of calcium ion (17), to qualitatively estimate whether the dissociation of calcium citrate increased as the temperature increased (Table 4). The small

TABLE 4

Apparent calcium ion concentration of calcium citrate solution

Solutions	O.D. 515 mμ	O.D. 470 mμ	515-470 values[a]	$[Ca^{++}]$	O.D. 515 mμ	O.D. 470 mμ	515-470 values[a]	$[Ca^{++}]$
		80° F.				200° F.[b]		
Standard								
1 m*M*/liter Ca	0.854	0.638	+0.22	1	0.629	0.469	+0.16	1
2 m*M*/liter Ca	0.796	0.745	+0.05	2	0.638	0.561	+0.08	2
3 m*M*/liter Ca	0.782	0.824	−0.04	3	0.629	0.602	+0.03	3
Test								
10 m*M* Ca + 9 m*M* citrate/liter	0.810	0.699	+0.11	1.6	0.653	0.505	+0.15	1.1
1.6 m*M* Ca/liter 0 citrate	0.796	0.699	+0.10	1.6	0.629	0.549	+0.08	2.0

[a] *Cf.* Reference 15.

[b] Murexide solutions fade at 200° F., but reproducible results were obtained by carefully controlling the heating times.

difference in observed optical density on heating indicates an increase in apparent calcium ion in the citrate-free solution (probably because heat-induced fading was slightly greater at 515 than at 470 mμ), but a decrease in the calcium citrate solution.

Relation of ultrafiltrate composition to heat stability. Since the changes induced by heat occur relatively rapidly (Figure 3), and are progressive with increasing temperature (Figure 4), it is reasonable to attempt to relate the effect of heating milk to 200° F. to its stability at higher temperature. Ultrafiltrates were, therefore, prepared from several bulk milks, and from a series of samples from each of four individual cows, at 80 and at 200° F., and the heat stability of the same samples was determined at 284° F. Two of the cows selected

had freshened only a few days before the first sampling, whereas the other two were nearing the end of their lactation period.

None of the attributes tested at either temperature (Table 5) showed any relation to the heat stability, and the differences between the 80 and 200° F. ultrafiltrates also failed to show any relation to heat stability. Grouping the samples in terms of their heat stability failed to indicate any relation between these groups and the composition of the ultrafiltrates. Of particular interest is the observation that even though the samples received four or five days after freshening of the cows had a high calcium ion content (19), they were not unstable in comparison to later samples of normal calcium ion content from the same cows.

DISCUSSION

Most of the results reported in this paper were obtained by the analysis of ultrafiltrates collected from hot milk, but cooled before analysis. Total calcium and phosphate in the ultrafiltrates would not, of course, be affected by the change in temperature after filtration, but interpretation of the pH and calcium ion results is more difficult.

The pH of phosphate buffers passes through a minimum at about 115° F., but would be essentially the same at the two temperatures (80 and 200° F.) used in these studies (1). Similar data were not found for citrate solutions. Data presented in the present paper indicate that the pH of ultrafiltrates may be about 0.1 unit higher at 180° F. than at room temperature. The pH value recorded for cooled samples of ultrafiltrate is, therefore, probably about 0.1 pH unit below that actually developed in the hot milk.

The only factor besides pH likely to affect calcium ion concentration during cooling appears to be citrate, and Evenhuis (5) has suggested that calcium dissociates to a greater extent at high temperature. To the best of the authors' knowledge, there is no evidence for this suggestion. The data presented herewith on the optical density of calcium murexide solutions indicate that no increase, but possibly a slight decrease, in calcium ion concentration occurs when calcium citrate solution is heated (Table 4). Thus, it appears probable that the calcium determined after cooling the ultrafiltrates is essentially the same as, or slightly higher than, that of the hot milk.

These considerations lead to the conclusions that heat affects the inorganic composition of milk serum to a much greater extent than has previously been demonstrated by analysis of recooled milks. When milk is heated to 200° F., approximately 50% of the soluble calcium and 18% of the soluble phosphate become nonultrafilterable, calcium ion concentration decreases by approximately 60%, and the acidity (H^+ concentration) increases at least twofold. On cooling, these changes are from 75 to 90% reversible.

Since similar changes were observed when artificial sera and solutions of calcium phosphate were separated from their precipitates at various temperatures (Tables 2 and 3), the primary cause appears to be a heat-induced change in the solubility and composition of the solid calcium phosphate. Association and dissociation of calcium caseinate and calcium citrate probably modify the effect of

TABLE 5

Composition and heat stability of raw skimmilks and composition of their ultrafiltrates collected at 80 and 200° F.

Date	Milk analysis 80°					Ultrafiltrate analysis								
						pH		Ca^{++}		Total Ca		PO_4		Citrate average
	pH	Total Ca	Total PO_4	Inorg. PO_4	Heat stability	80° F.	200° F.	80° F.	200° F.	80° F.	200° F.	80° F.	200° F.	
Bulk milks		(mM/liter)			(min.)			(mM/liter)						
Dec. 9, 1957	6.47	31.6	39.1	20.7	13.3	6.57	6.03	3.8	1.4	8.8	5.0	11.7	10.0	8.8
Dec. 11, 1957	6.57	30.0	30.9	19.1	10.3	6.72	6.13	3.8	1.3	9.0	4.9	10.1	7.8	8.5
Dec. 16, 1957	6.63	30.0	29.1	19.9	8.5	6.72	6.26	2.5	1.1	7.6	3.5	10.2	8.0	7.7
Dec. 18, 1957	6.58	25.8	30.2	19.2	9.0	6.73	6.18	2.8	1.2	8.2	4.5	10.2	8.7	8.7
Jan. 1, 1958	6.59	25.0	27.5	18.6	11.0	6.80	6.19	2.6	1.1	8.5	4.4	10.0	8.2	8.3
Jan. 8, 1958	6.62	24.2	27.8	17.8	9.5	6.77	6.26	2.9	1.2	7.7	3.9	9.9	8.5	8.3
Jan. 13, 1958	6.63	24.8	27.2	18.0	11.6	6.77	6.18	2.7	1.0	7.7	3.8	10.5	8.5	8.1
Jan. 15, 1958	6.60	24.8	27.9	19.4	11.9	6.68	6.10	3.0	1.2	8.7	4.3	10.4	8.1	8.0
Cow No. 1, freshened November 27, 1957														
Dec. 2, 1957	6.54	30.6	38.1	20.3	8.3	6.72	6.06	4.4	1.6	9.7	5.8	9.3	7.1	7.6
Dec. 16, 1957	6.60	28.2	28.8	18.4	9.1	6.82	6.17	2.6	0.8	6.2	4.1	8.9	6.7	5.6
Dec. 23, 1957	6.60	25.4	29.7	.....	6.5	6.72	6.23	2.6	1.3	8.2	5.6	9.9	8.8	8.6
Dec. 30, 1957	6.57	28.1	27.5	17.8	5.6	6.76	6.26	3.1	1.5	8.6	5.7	9.3	7.7	8.6
Jan. 6, 1958	6.62	24.5	27.5	18.8	6.3	6.82	6.27	2.8	1.3	7.3	4.7	9.8	8.7	8.4
Jan. 13, 1958	6.62	24.4	27.9	18.7	6.0	6.86	6.21	2.7	0.8	8.3	4.4	10.6	8.3	8.0
Cow No. 2, freshened November 28, 1957														
Dec. 2, 1957	6.40	30.2	35.5	20.0	6.0	6.54	5.94	5.3	1.8	11.1	6.4	10.2	8.1	9.0
Dec. 16, 1957	6.55	28.6	32.0	19.6	5.3	6.70	6.18	2.7	0.9	9.0	4.5	10.5	.....	7.9
Dec. 30, 1957	6.60	27.3	28.5	19.5	6.3	6.76	6.26	2.9	1.4	8.8	4.8	10.4	7.5	9.2
Jan. 6, 1958	6.58	26.3	27.1	18.6	5.8	6.80	6.22	3.0	1.4	8.9	5.7	9.2	7.1	9.9
Jan. 13, 1958	6.59	26.7	27.9	18.7	8.1	6.78	6.14	3.0	1.0	10.3	4.5	10.2	7.8	8.8
Cow No. 3, freshened March 20, 1957														
Dec. 11, 1957	6.69	31.7	28.0	17.3	5.1	7.00	6.40	3.0	1.2	6.5	3.5	7.0	5.4	6.4
Dec. 18, 1957	6.71	24.0	26.0	18.1	3.8	7.12	6.55	2.5	0.8	5.8	3.0	7.2	5.7	6.3
Jan. 8, 1958	6.81	31.0	27.5	17.2	3.3	7.16	6.56	2.7	1.1	5.3	2.7	7.7	4.7	6.0
Jan. 15, 1958	6.59	28.2	27.4	17.9	7.1	7.01	6.44	2.7	1.0	5.3	3.2	6.8	5.5	5.8
Cow. No. 4, freshened March 20, 1957														
Dec. 11, 1957	6.61	26.8	28.4	18.7	5.5	6.79	6.32	2.7	1.0	6.5	3.6	10.6	8.8	7.5
Dec. 18, 1957	6.59	26.0	28.2	20.1	5.0	6.80	6.30	2.4	0.9	7.1	3.9	11.7	10.3	7.8
Jan. 8, 1958	6.60	25.9	30.3	20.2	4.8	6.80	6.30	2.6	1.0	6.6	3.4	11.9	10.4	7.9
Average	6.59[a]	27.3	29.5	18.9	7.4	6.79[a]	6.21[a]	3.0	1.2	7.9	4.4	9.4	7.7	7.9

[a] Average H^+ concentration reconverted to pH.

heat on the composition of the ultrafiltrate, but there is no reason to assume that they play a major role in determining the extent of the changes.

On the basis of analyses made on cooled samples, Pyne and McHenry (15) concluded that only a small portion of the heat-induced decrease in pH resulted from precipitation of calcium phosphate, and that most of the pH change was caused by decomposition of lactose and casein phosphate ester. However, formation of acid from these sources would be expected to occur relatively slowly, particularly at temperatures below the boiling point, and probably accounts for only a small part of the rapid pH change demonstrated by the hot ultrafiltration technique.

Both Evenhuis (5) and Pyne and McHenry (15) have suggested that calcium ion concentration[1] in hot milk should be higher than that of cold milk: Evenhuis assumed an increased dissociation of calcium citrate with increasing temperature, and Pyne and McHenry based their suggestion on the assumption that the dominant effect would be that induced by increasing acidity. However, the data presented herewith show that there is a marked decrease in calcium ion concentration as milk is heated, and it therefore appears that the dominant effect is the decreased solubility and altered composition of calcium phosphate. Explanations of the heat coagulation of milk colloids based on an assumed increase in calcium ion activity (5, 6, 15) are, therefore, unacceptable.

Analyses of ultrafiltrates obtained from cold and hot milk have not provided any indication of the factors controlling heat stability, but make it appear improbable that changes in the inorganic composition of the serum as the milk is heated are a major factor. The extent to which calcium combines with casein may be important, but the assumption that a constant amount of inorganic calcium is attached to casein (3) is not acceptable if the calcium ion activity and the pH are not constant, and Evenhuis' conclusion (7) that there is a constant $\frac{Ca + Mg}{P}$ ratio in the precipitate requires further proof, in view of the known variability of calcium phosphate precipitates (2, 4) and the observed effect of heat on the composition of the precipitate from calcium phosphate solutions (Table 3). Calculation of the caseinate calcium is, therefore, not possible at present.

[1] Evenhuis (5) uses the term activity but, from the context [e.g., "The calcium ion activity of milk was estimated by Smeets by the purpurate method" (5) p. 228], it is apparent that he uses the term as a synonym for concentration.

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TURBIDIMETRIC MICRO-DETERMINATION OF MAGNESIUM IN MILK[1]

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SUMMARY

From 5 to 40 μg. of magnesium can be determined by measuring the turbidity formed when the potassium salt of erucic acid is added in the presence of potassium oxalate. Preliminary removal of calcium as oxalate is achieved rapidly at room temperature; other milk components do not interfere. Analysis of various samples of milk and serum by the present technique showed that, compared with ashed samples, direct analysis gave results which agreed within ± 3.3%. The method is simple, convenient, and suitable for routine analysis.

Available methods for magnesium determination have serious limitations for use on small samples of synthetic or natural milk serum. Gravimetric methods are time-consuming (4), colorimetric procedures based on the adsorption of dye by magnesium hydroxide are oversensitive to ionic interference (7), the ethylenediaminetetraacetate titration lacks sensitivity (1, 3, 8), and flame spectrophotometry requires a rigid balancing of all cations in the test and standard solutions (10). Grégoire and Sola's turbidimetric method (2) is of limited value because of the relatively high solubility of magnesium oleate in dilute salt solutions.

Preliminary tests with a potassium erucicate reagent indicated that this compound reacted with magnesium to form turbidity which, in the presence of oxalate ions, was relatively unaffected by salt concentrations similar to those found in milk sera. A method based on this reaction is described below.

RECOMMENDED METHOD

Reagents. Glass-redistilled water is used throughout. All reagents are stored at room temperature in polyethylene ware.

Potassium Erucicate:

Stock solution: Dissolve 2.0 g. of reagent-grade potassium hydroxide in 6.0 ml. of water and add 60 ml. of 95% ethyl alcohol. Add 8.5 g. of erucic acid (practical grade, supplied by Brickman and Co., Montreal, Canada) and reflux gently for about 1 hr., then dilute to 250 ml.

Reagent: Immediately before using, add 1.0 ml. of concentrated reagent-grade ammonium hydroxide to 6.0 ml. of stock erucicate solution and dilute to 100 ml.

Potassium Oxalate:

Solution A: Prepare a 30% w/v solution of reagent-grade potassium oxalate monohydrate in water.

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[1] Issued as N.R.C. No. 5221.

Solution B: Add 2.0 ml. of potassium oxalate Solution A to 25 ml. of water.

Standard Magnesium Solution:

Dissolve 10.1353 g. of reagent-grade magnesium sulfate heptahydrate in water to which 1.0 ml. of concentrated hydrochloric acid has been added, and dilute to one liter. This solution contains 1.00 mg. of magnesium per milliliter and is used to prepare suitable standards.

Procedure. To an aliquot of test solution having a pH between 4.0 and 9.0, and diluted to contain 5 to 40 μg. of magnesium per milliliter, add 8% v/v of potassium oxalate Solution A with constant stirring. Allow the solution to stand at room temperature for at least 20 min., then centrifuge 2 min. at approximately 1,000 $\times$ g. Then add, to separate colorimeter tubes:

a) 1.0 ml. of the decalcified solution obtained above, followed by 4.0 ml. of water.

b) An aliquot of standard magnesium solution containing between 5 and 40 μg. of magnesium, followed by 1.0 ml. of potassium oxalate Solution B, then water to bring the volume to 5.0 ml.

c) 1.0 ml. of potassium oxalate Solution B, followed by 4.0 ml. of water (this serves as a reagent blank).

To all tubes, add 5.0 ml. of potassium erucicate reagent and swirl briskly. Allow the samples to stand at room temperature for at least 1.5 hr., then read at 400 mμ with an Evelyn (or similar) colorimeter, setting the reagent blank at 100% transmission. Determine the magnesium content of the test solution by reference to the standards.

If the solution to be analyzed is turbid (e.g., diluted skimmilk), prepare a sample blank as follows: To a duplicate aliquot of decalcified test solution, add 5.0 ml. of a 1/100 dilution of concentrated ammonium hydroxide (to simulate the alkalinity of the test) and make to 10.0 ml. with water. Read this sample at 400 mμ against a water blank and subtract its optical density from that of the test solution.

EXPERIMENTAL PROCEDURE

Removal of calcium. The effect of calcium on the erucicate method was investigated at various magnesium levels. It was found that 5 μg. or less of calcium in the sample did not interfere in the determination, but that larger amounts caused high recoveries at the lower magnesium levels (Figure 1). Preliminary removal of calcium was, therefore, essential.

Most techniques for the precipitation of calcium recommend the use of ammonium oxalate or of oxalic acid and alkali, added at high temperature (4). However, it appeared possible that the more soluble potassium oxalate could be used (to avoid excessive dilution of the test solution) and that satisfactory precipitation of calcium could be achieved at room temperature.

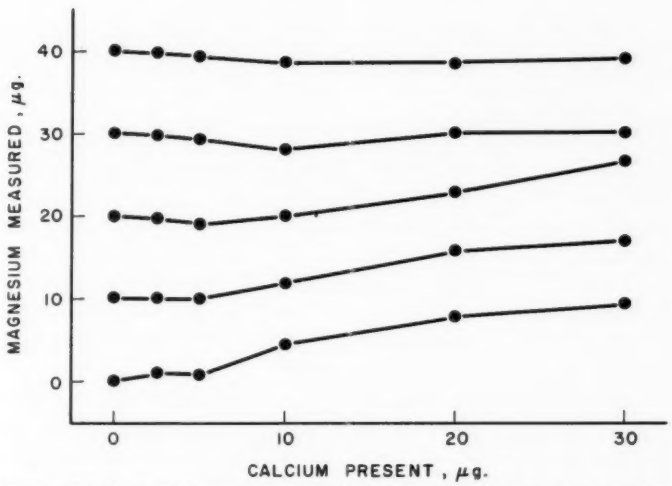

FIG. 1. Effect of calcium on the turbidimetric determination of magnesium.

The effect of potassium oxalate concentration on the precipitation of calcium from a solution containing calcium, magnesium, phosphate, and citrate in concentrations similar to those found in milk sera was studied at 25° C. Additions of 2, 4, 8, or 12 ml. of 30% potassium oxalate solution were made to 100-ml. aliquots of the synthetic serum. After 20 min., the samples were centrifuged and the precipitates dissolved in dilute hydrochloric acid and analyzed for calcium (6). The supernatants were analyzed for magnesium, making sure that the aliquots introduced at least 30 μg. of magnesium per test to avoid possible interference by residual calcium. Results (Table 1) showed that mag-

TABLE 1

Effect of potassium oxalate concentration on precipitation of calcium at room temperature, and on recovery of magnesium in the supernatant[a]

Milliliters of 30% potassium oxalate added to 100 ml. of solution	Calcium precipitated		Magnesium in supernatant	
	μg. per ml.	% found	μg. per ml.	% found
2	352.5	89.5	128.0	98.5
4	371.3	94.2	126.3	97.2
8	393.8	99.9	127.0	97.7
12	397.5	100.9	131.7	101.3

[a] All solutions contained 394 μg. of calcium and 130 μg. of magnesium per milliliter (as the chlorides), and 10 m*M* K_2HPO_4, 10 m*M* Na_2SO_4, 8 m*M* $Na_3Citrate$, and 32 m*M* KCl per liter, adjusted to pH 5.20.

nesium was apparently not coprecipitated by any of the oxalate treatments; however, an 8% addition of potassium oxalate was required for complete calcium removal.

The amount of residual calcium, after precipitation with the 8% v/v addition of stock potassium oxalate solution, was determined with solutions containing

from 10 to 400 μg. of calcium per milliliter but containing no magnesium. Residual calcium concentrations varied nonsystematically between 0 and 4.2 μg. per milliliter, averaging 1.7 μg. per milliliter, indicating that the precipitation procedure was fully adequate.

The effect of pH on the efficiency of precipitation of calcium oxalate and subsequent recovery of magnesium in the supernatant were studied with solutions containing 40 to 1,200 μg. of calcium and 24 or 240 μg. of magnesium per milliliter, adjusted to pH values between 2 and 9. Values greater than 9 were not studied, because precipitation of magnesium hydroxide might occur. Within the range 4 to 9, pH did not affect precipitation of calcium or recovery of supernatant magnesium. Between 2.5 and 4.0, magnesium coprecipitated with calcium when the concentration of magnesium in the initial solution was low. Below pH 2.5, calcium was incompletely precipitated.

Formation of the magnesium erucicate turbidity. The effect of amount of erucic reagent on the development of turbidity was studied with magnesium standards by adding 5 ml. of various dilutions of the stock erucicate solution per test. The sensitivity of the method varied with the concentration of erucicate and with the magnesium level (Figure 2), but this variation was minimal in the

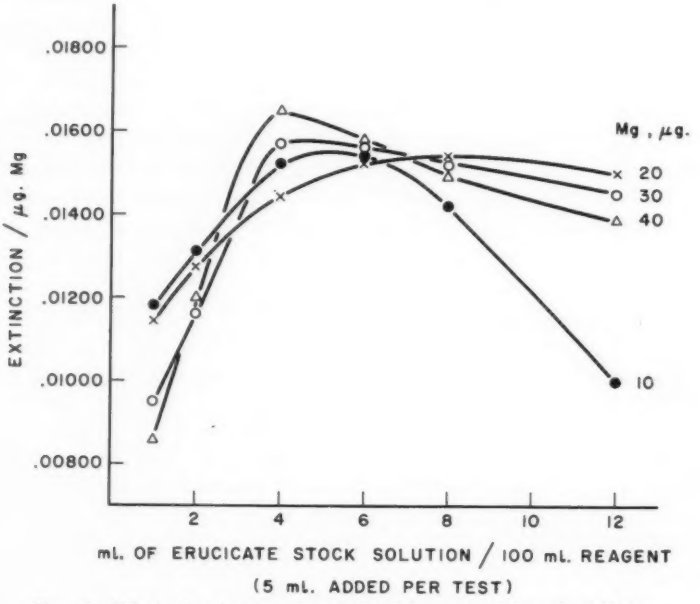

FIG. 2. Effect of potassium erucicate level on formation of turbidity.

region of the 6% dilution of the stock erucicate solution. This level was, therefore, adopted and, since the diluted erucicate tended to destabilize after a few days, fresh dilutions were prepared as required. Practical-grade erucic acid

is recommended and, although similar results were obtained with reagent-grade erucic acid, the use of the coarser grade resulted in improved tolerance for various ions.

Development of turbidity with magnesium was gradual and, even after 2 hr. at room temperature, it continued to develop at the rate of 2% per hour (Figure 3). The reagent blanks remained clear for about 6 hr. A waiting period of

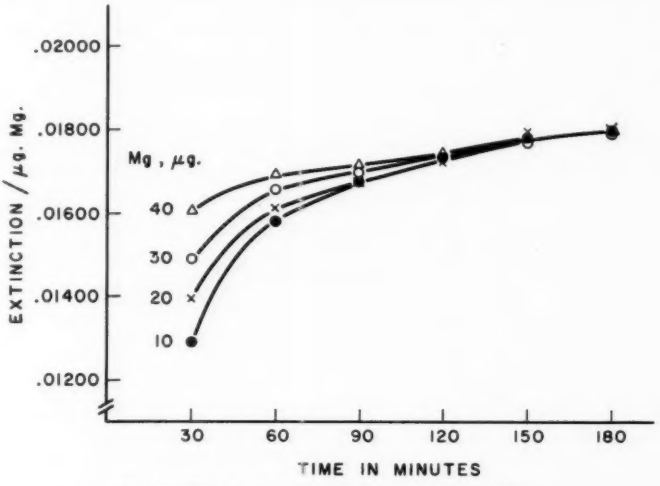

FIG. 3. Effect of time on formation of turbidity.

1.5 to 2 hr. before reading gave results which were easily reproducible. The extinction coefficient per μg. of magnesium was constant at concentrations greater than 10 μg. per test; however, at the lower levels, it was necessary to use standards having a magnesium content close to that of the unknown. The preparation of at least four standards, covering the range 5 to 40 μg. of magnesium, is recommended with each series of determinations.

The turbidity that formed in the presence of magnesium increased its absorption of light when the wave-length was varied from 600 to 250 mμ (Beckman DU spectrophotometer). However, the blank absorption gradually increased at wave lengths below 400 mμ. It was, therefore, recommended that readings should be made at 400 mμ.

The effect of temperature was studied at various constant levels between 60 and 90° F. It was found that sensitivity increased gradually (by about 1% per ° F.) as the temperature was raised from 60 to 85° F., but that temperatures above 85° resulted in erratic development of turbidity and should be avoided. Provided that the room temperature is not subject to drastic fluctuations, and that standards are prepared with each group of analyses, no special precautions need to be taken to control temperature.

The degree of turbidity was unaffected by variations in final pH between 10.4 and 11.1. At a final pH below 10.4, erratic development of turbidity occurred. Final pH values higher than 11.1 were not studied. Inclusion of concentrated ammonium hydroxide in the erucicate reagent insured the correct pH for samples of milk and serum.

Effect of various ions. The removal of calcium with potassium oxalate introduces about 20 mg. of the oxalate salt (anhydrous basis) with a 1-ml. aliquot. Additions of potassium oxalate to magnesium standards showed (Figure 4) that

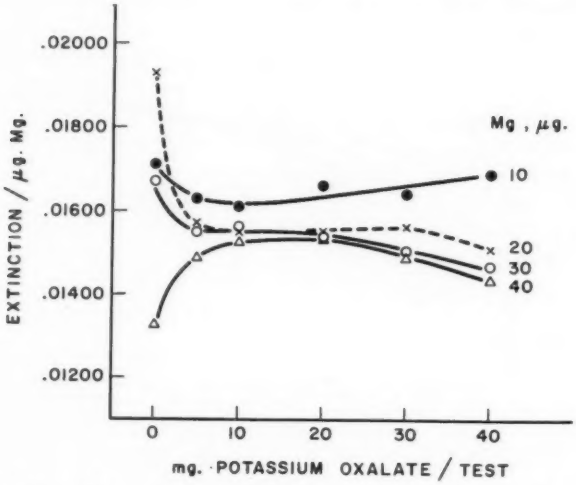

FIG. 4. Effect of potassium oxalate on formation of turbidity.

the presence of this salt at concentrations between 5 and 30 mg. per test gave a relatively uniform turbidity development over the range of recommended magnesium levels. Potassium oxalate also increased the tolerance of the erucicate reagent for other salts (e.g., in the absence of oxalate, 5 mg. of sodium sulfate caused extensive precipitation of the reagent, but the addition of 20 mg. of potassium oxalate per test completely prevented this undesirable reaction). Addition of oxalate (20 mg. per test as the potassium salt) to solutions which have not been treated for removal of calcium is, therefore, recommended. If less than 1 ml. of decalcified solution is used, the oxalate level should be adjusted to the recommended concentration.

The effect of chlorides, sulfates, phosphates, or citrates of potassium and sodium on the determination of magnesium in standard solutions also was studied. The potassium oxalate level was kept constant at 20 mg. per test. Additions of 90 μ eq. or less of potassium and of 50 μ eq. or less of sodium per test had no effect on the method, regardless of the nature of the anion. Greater additions of either potassium or sodium tended to decrease formation of turbidity. The tolerance for these salts enables satisfactory analyses to be performed on milk

or serum, in which the concentration of potassium and sodium is about 40 and 20 μ eq. per ml., respectively.

APPLICATION

Accuracy of the method was estimated by analyzing solutions containing magnesium, calcium, phosphorus, potassium, sodium, sulfate, and chloride ions, adjusted to ionic strength 0.08 and pH 5.2. The concentration of magnesium and calcium was varied between 4 and 10 mM per liter, that of phosphorus between 4 and 15 mM per liter; whereas, that of the various other ions was varied so as to keep the ionic strength and pH constant. The results of single analyses of ten of these solutions averaged 98.6 $\pm$ 1.5% of the expected values. In routine analysis, agreement between duplicate determinations has been within $\pm$ 3%.

Two samples of skimmilk, and various sera prepared from them, were analyzed with and without preliminary ashing. Values obtained for the sera were multiplied by 0.97 to correct for the volume change caused by removal of protein. Skimmilk and hydrochloric acid serum appeared to contain the same amount of magnesium (Table 2), but results with rennet serum and ultrafiltrate were

TABLE 2
Analysis of skimmilk and various types of milk serum

	Milk 1			Milk 2		
	Magnesium, μg/ml		Direct %	Magnesium, μg/ml		Direct %
	Direct	Ashed	Ashed	Direct	Ashed	Ashed
Skimmilk	103.0	98.0	105.1	79.5	83.5	95.2
HCl serum[a]	105.4	99.2	106.3	83.3	84.1	99.0
Rennet serum[b]	79.9	83.7	95.5	59.9	64.8	92.4
Ultrafiltrate[c]	72.3	66.9	108.1	57.7	56.3	102.5
		Av.	103.8		Av.	97.3

[a] Prepared by adding a 6% v/v amount of 1.0 N hydrochloric acid to skimmilk and filtering out the coagulated casein.
[b] Obtained by Ling's procedure (5).
[c] Prepared according to Tessier and Rose (9).

decidedly lower. The magnesium level found in ultrafiltrate was approximately two-thirds of that found in skimmilk, a result which agrees with the findings of White and Davies (11). The nonsystematic variation between results of ashed and nonashed samples averaged approximately $\pm$ 3.3%.

Our studies have shown that, with the exception of calcium, the components present in ashed samples of milk and serum do not interfere with the determination. The absence of a systematic difference between results with ashed and nonashed samples indicates, therefore, that the other components of milk and serum do not interfere significantly, and that the direct analysis of normal milk and serum by the turbidimetric method can be recommended.

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EFFECT OF VARIOUS SALTS ON THE COAGULATION OF CASEIN

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SUMMARY

Addition of potassium or sodium chloride (80 or 130 m*M*) to skimmilk increased the time the milk could be held in frozen storage without development of 4% precipitate by volume. Addition of phosphate or calcium (10 m*M*) had the opposite effect. Addition of large amounts of sodium chloride (up to 1.0 *M*) offset the destabilizing effect of phosphate or calcium at 0, 10, and 20° F., but addition of similar amounts of potassium chloride stabilized the samples only at 10 and 20° F.

Addition of potassium chloride (1.0 *M* or more) and of phosphate (100 m*M* or more) induced gelation of milk at room temperature. Addition of sodium chloride and phosphate did not induce gelation. Addition of either potassium or sodium chloride (2.3 *M*) shifted approximately 20% of the insoluble calcium and 7.5% of the insoluble phosphate to the soluble forms, displaced calcium from casein, and increased the dissociation of calcium citrate. A possible explanation of these effects, based on the interlinking of potassium-calcium-caseinate micelles by precipitating calcium phosphate, is presented.

Variations in the concentration of certain inorganic salts or ions, particularly calcium, are known to affect the coagulation of casein in abnormal milk (2, 14), in milk treated with rennet or other coagulating enzymes (10), and in frozen milk (3, 13). The unfrozen portion of milk stored at −7.5° C. contains approximately 0.8 *M* salt (ten-fold concentration) prior to lactose crystallization (15), and it can be estimated that up to a 30-fold concentration develops as lactose crystallizes. Coagulation of the casein occurs as this high concentration of salt develops (17, 18), but it is not known whether total salt concentration or the concentration of specific components induces the coagulation.

Under conditions that do not cause salt crystallization, the concentration of a component in the unfrozen portion of frozen milk depends directly upon its initial concentration and the total amount of water retained in the unfrozen state by all components at the storage temperature used. Therefore, addition of a salt that promotes casein coagulation should decrease the storage life of milk. Addition of a solute that does not promote coagulation will dilute the other components by retaining more unfrozen water and, thus, should increase storage life. Glycerol, sucrose, and dextrose (1, 13, 20) and hydrolyzed lactose (18) probably act mainly in this manner. This paper presents the results of a study of the effect of added salts on casein coagulation.

MATERIALS AND METHODS

Milk obtained from morning milkings at the Central Experimental Farm, Ottawa, was separated and pasteurized at 150° F. for 30 min. Storage tests were carried out as previously described (13), i.e., samples were frozen in 15-ml. vials and, at suitable intervals (depending on the temperature), single vials were thawed at 40° F. overnight and the contents centrifuged at *ca.* 500 × g. for

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10 min. The graphically interpolated time needed to develop 4% precipitate by volume is reported as the storage life. Analytical methods were: calcium ion in ultrafiltrate (16), total calcium (8), phosphate (9). Ashing was done in platinum crucibles at 550° C. for 15 hr.

Calcium caseinate suspensions were prepared by dispersing 3 g. of casein prepared by Van Slyke and Baker's method (19) in approximately 95 ml. of saturated calcium hydroxide solutions and adjusting the pH to 6.6 to 6.7 with hydrochloric acid. Sufficient quantities of molar solutions of ammonium phosphate, ammonium citrate, and magnesium chloride were then added to give concentrations of phosphate, citrate, and magnesium (30, 9, and 5 m*M*/l, respectively) comparable to those in milk. One per cent glycerol was added to give the freezing point depression of normal milk while avoiding the possibility of lactose crystallization. These preparations were essentially sodium- and potassium-free.

"Redispersed casein" was casein-calcium-phosphate complex of fresh skimmilk sedimented at 60,000 × g. (Spinco Model L, No. 30 head, 30,000 r.p.m., 1 hr.) and redispersed by trituration into a solution containing calcium, phosphate, citrate, and magnesium, added as the ammonium and chloride salts, in approximately the concentrations found in normal milk. One per cent glycerol was added in lieu of 4% lactose.

Since samples of milk can differ markedly in their storage life when frozen, and in other characteristics, data for single tests are reported in the tables. However, replicate (duplicate or more) tests done under the stated conditions, and tests done under varied conditions not reported in detail, confirmed the direction and relative magnitude of the changes reported.

RESULTS

Freezing tests with one salt added. Small additions (up to 40 m*M*) of calcium or phosphate sharply decreased the storage life of frozen milk at all temperatures studied (Table 1). The destabilizing effect of calcium was greater than that of phosphate, but appeared to be relatively independent of temperature. The destabilizing effect of phosphate was less at 0° F. (−18° C.) than at 20° F. (−7° C.). Similar effects were observed in calcium caseinate and in redis-

TABLE 1

Effect of added calcium and phosphate on the storage life of frozen skimmilk[a]

Addition	Storage life *(days)*		
	20° F.	10° F.	0° F.
None	35	40	50
2.5 m*M* Calcium	35	20	30
5.0 m*M* Calcium	5	4	2
10.0 m*M* Calcium	3	2	2
2.5 m*M* Phosphate	35	25	50
5.0 m*M* Phosphate	8	18	50
10.0 m*M* Phosphate	2	8	30

[a] All samples adjusted to the pH of the control, pH 6.53, before freezing.

persed casein when calcium or phosphate was added in excess of the amounts found in normal milk. The converse effect has been observed upon removal of calcium (3, 4). Large additions of either calcium or phosphate at near-normal levels of the other could not be studied because of the limited solubility of calcium phosphate salts.

Additions of either potassium or sodium chloride to skimmilk or to redispersed casein had a definite stabilizing effect during frozen storage at all temperatures studied (Table 2). The effect of either salt was more marked at high than at low storage temperatures.

TABLE 2
Effect of added sodium and potassium chlorides on the storage life of frozen skimmilk

Addition	Storage life *(days)*		
	20° F.	10° F.	0° F.
Skimmilk			
None	25	32	63
130 m*M* Sodium chloride	80	57	80
80 m*M* Potassium chloride	58	41	80
Redispersed casein			
None	2	2	16
130 m*M* Sodium chloride	35	18	32
80 m*M* Potassium chloride	29	12	17

Freezing tests with two salts added. If sodium and potassium chlorides exert this stabilizing action by reducing the concentration of calcium and phosphate in the unfrozen portion of frozen milk, then larger additions of these salts should offset the destabilizing effect of added calcium or phosphate. To test this possibility, up to 1 *M* of sodium or potassium chloride was added to milks containing up to 20 m*M* added calcium and 40 m*M* added phosphate.

The results obtained with sodium chloride indicate that it was possible to offset completely the destabilizing effect of added calcium (Figure 1A) or phosphate (Figure 1B), at all storage temperatures studied. On the other hand, potassium chloride offset the effect of added calcium at 20° F. (−8° C.), and partially offset it at 10° F. (−12° C.), but decreased storage life of two samples and the control, at 0° F. (−18° C.) (Figure 2A). Also, potassium chloride offset the effect of added phosphate only partially at 20 and 10° F., and did not stabilize any sample with added phosphate at 0° F. (Figure 2B). The inability of high concentrations of potassium chloride to offset the effects of added calcium or phosphate at 0° F. may be explained by the assumption that crystallization of the potassium chloride occurred because eutectic conditions were exceeded.

It is apparent in Figures 1 and 2 that there was some tendency for the lowest level (0.1 *M*) of sodium or potassium chloride to increase the destabilizing effect of added calcium or phosphate. A possible explanation of this is given under Discussion.

Tests without freezing. Preliminary tests had indicated that addition of potassium chloride and a neutral phosphate solution to milk caused the casein

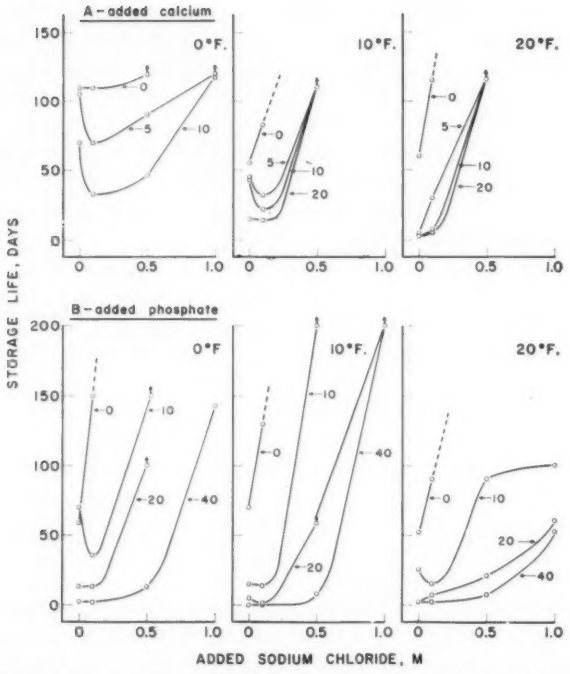

FIG. 1. Effect of added sodium chloride on storage life of frozen skimmilk containing added calcium or phosphate. Figures on curves indicate concentration, in m*M*, of added calcium (A) or phosphate (B). Arrows attached to points indicate that the last sample, taken at the time shown, had not coagulated. Each set of tests (four curves) was from a separate sample of milk.

to gel within a few hours at room temperature. The rate of gelation varied between samples of milk, but within any one sample consistent results were obtained.

Gelation could be induced by addition of phosphate to milk containing 1 *M* or more added potassium chloride per liter, and gelation time decreased progressively with increasing potassium chloride concentration. When 2.3 *M* potassium chloride was present, addition of 240, 160, and 120 m*M* phosphate per liter caused gelation in .5, 2.5, and 4 hr., respectively. Increasing the lactose concentration delayed gelation slightly; increasing citrate delayed it markedly. At a fixed potassium chloride and phosphate level, gelation time increased with decreasing pH and with decreasing temperature.

A similar gelation was obtained with redispersed casein preparations, provided calcium, phosphate, and potassium chloride were included in the resuspending medium or added to the suspension. Conditions leading to the formation of a calcium phosphate precipitate appeared to be a prerequisite for gel formation. It was also possible to cause gelation in skimmilk by acidifying it

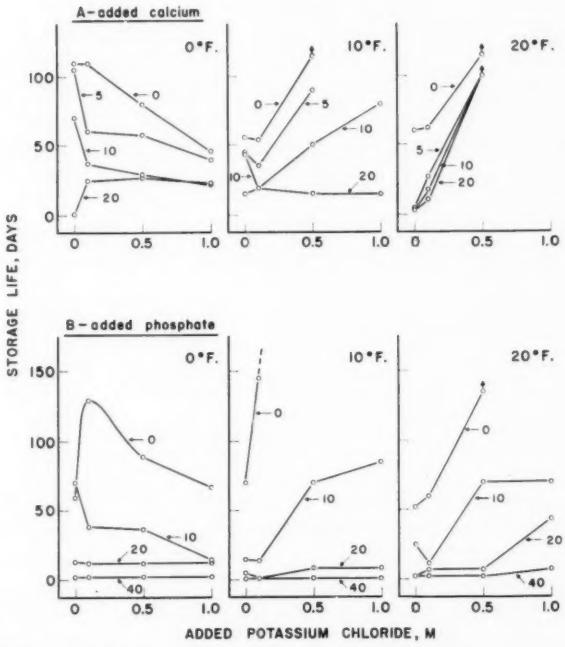

FIG. 2. Effect of added potassium chloride on storage life of frozen skimmilk containing added calcium or phosphate. Details as in Figure 1.

to pH 5.2, adding 160 m*M* phosphate, then neutralizing so as to induce extensive precipitation of calcium phosphate.

Gelation could not be induced either in skimmilk or in casein resuspensions by the addition of phosphate and sodium chloride. Addition of sodium chloride to a milk containing 160 m*M* added phosphate and 1.5 *M* potassium chloride delayed gelation; aliquots with 0, 0.8, and 1.5 *M* added sodium chloride gelled in 100, 180, and 900 min., respectively.

Addition of sodium chloride to milk caused a greater increase in acidity than did addition of potassium chloride (Table 3), but addition of sufficient sodium hydroxide to maintain the original pH did not promote gelation in the presence of phosphate and sodium chloride.

To determine what effect added sodium and potassium chlorides had on the distribution of salts in milk, ultrafiltrates [obtained as in (16)], and the supernatant and sedimented solids obtained by centrifuging at 60,000 × g., were analyzed. Analysis of the ultrafiltrates (Table 3) showed that added potassium or sodium chloride caused a marked increase in calcium ion, total calcium, and total phosphate. Even with sodium chloride, the increase in calcium ion concentration was more than three-fold that caused by addition of hydrochloric acid to give the same pH. Total ultrafiltrable calcium and phosphate appeared to

TABLE 3
Analysis of ultrafiltrates from skimmilk with added salt or acid

Compound added	Concentration added	Final pH	Ca^{++}	Total calcium	Phosphate
	(M)		*(mM)*	*(mM)*	*(mM)*
Potassium chloride	0	6.67	2.7	8.2	8.6
	0.17	6.62	4.4	9.5	9.8
	0.42	6.61	6.3	10.6	9.6
	0.92	6.60	7.2	11.4	10.2
	1.42	6.60	8.0	11.1	10.2
	1.92	6.60	8.2	11.6	10.1
Sodium chloride	0.17	6.50	4.9	9.4	9.5
	0.42	6.50	7.2	10.7	10.0
	0.92	6.42	8.4	11.1	10.7
	1.42	6.37	9.0	11.1	10.5
	1.92	6.32	10.0	12.0	10.4
Hydrochloric acid	.....	6.55	3.2	8.6	9.7
	.....	6.32	4.6	9.3	10.3
	.....	6.09	7.2	11.7	12.0
	.....	5.86	9.7	12.8	13.7

increase with additions of up to 1.0 *M* of the chlorides, but there was little change with further additions. Since the increase in total calcium is not sufficient to account for the increase in calcium ion, it appears that excess sodium or potassium increased the dissociation of calcium citrate.

Analysis of the sera and solids (Table 4) showed that addition of 2.3 *M* potassium chloride shifted approximately 23% of the insoluble calcium and 8% of the insoluble phosphate into the soluble form; whereas, addition of 2.3 *M* sodium chloride shifted 17 and 7%, respectively. The total (solids plus serum) calcium and phosphate found by these analyses agreed within 3% of the value obtained by analysis of the original skimmilk. Assuming that the shift in phosphate represents solution of tricalcium phosphate (molar Ca/P ratio, 1.5:1), 1.5 to 1.8 m*M* (i.e., 1.5 × average change in phosphate) of calcium will have dissolved from this source, and the remaining 1.4 to 2.8 m*M* must have been dis-

TABLE 4
Effect of added sodium and potassium chloride on the composition of milk serum and milk solids[a]

Component	Concentration *(mM)*			Change caused by	
	Control	After KCl addition	After NaCl addition	KCl	NaCl
Calcium in serum	7.9	12.3	11.6	+4.4	+3.7
Calcium in solids[b]	18.4	14.2	15.7	−4.2	−2.7
Totals	26.3	26.5	27.3		
Calcium in original skimmilk[b]	26.9				
Phosphate in serum	10.6	11.8	11.2	+1.2	+0.6
Phosphate in solids[b]	14.8	13.6	13.5	−1.2	−1.3
Totals	25.4	25.4	24.7		
Phosphate in original skimmilk[b]	25.8				

[a] 2.3 *M* salt added, pH 6.61.
[b] Skimmilk and solids analyzed after dry ashing; results expressed as m*M* in original milk.

placed from casein by the added sodium or potassium. The greater increase in soluble calcium obtained with added potassium than with added sodium chloride probably indicates a greater displacement of calcium from casein by the smaller (less hydrated) potassium ion, but may be within the experimental error.

Addition of 160 m*M* phosphate precipitated most of the soluble calcium from normal milk and from milk in which the soluble calcium content had been increased by addition of sodium or potassium chloride (Table 5). Therefore,

TABLE 5

Effect of added chlorides and phosphate on the composition of milk ultrafiltrate

Treatment	Concentration (*mM*)	
	Calcium ion	Total calcium
Control, no addition	2.4	7.1
Added 2.3 *M* KCl	5.1	12.0
Added 2.3 *M* NaCl	7.2	11.3
Added 160 m*M* phosphate	0.4[a]	3.4
Added 2.3 *M* KCl and 160 m*M* phosphate	0.4[a]	2.9
Added 2.3 *M* NaCl and 160 m*M* phosphate	0.2[a]	1.8

[a] These values are below the range of accuracy of the calcium ion method.

considerably more calcium phosphate must have precipitated when 160 m*M* phosphate was added to milk containing added potassium or sodium chloride than when the same amount of phosphate was added to skimmilk.

DISCUSSION AND CONCLUSIONS

The results presented in this paper can be explained on the hypothesis that casein gels and the easily dispersed, flocculent coagula frequently observed during the early stages of frozen storage are developed by the interlinking of mixed calcium-potassium caseinate by precipitating calcium phosphate. The calcium phosphate present in normal milk is presumably insufficient to induce gelation by itself, but Pyne (11) has shown that phosphate accelerated rennet coagulation of calcium caseinate sols, and decreased the coagulation time of milk at 130° C. (12). Fresh calcium phosphate precipitates are known to adsorb on to microorganisms (6, 7) and on to casein (5, 6, 7, 11, 21), so could conceivably interlink casein micelles. Zittle *et al.* (21) have shown that phosphate increases the degree of aggregation in calcium caseinate sols at 25° C.

High levels of potassium chloride displaced calcium from casein and citrate, increased the solubility of calcium phosphate (Tables 3 and 4), and increased the amount of fresh calcium phosphate formed on addition of phosphate (Table 5). Sodium chloride behaved similarly as far as could be detected by chemical analysis, but presumably differences in the physical characteristics (e.g., degree of hydration) of potassium and sodium caseinate prevented gel formation in the presence of excess sodium. In other words, mixed calcium-potassium caseinate readily interlinked to form a gel, but mixed calcium-sodium caseinate did not.

During freezing, added potassium and sodium chloride apparently exerted two opposite effects: They increased calcium and phosphate precipitation by bringing more calcium into solution, and they decreased precipitation by retaining additional water in the unfrozen state. The shape of the curves of Figures 1 and 2 suggests that in the presence of added calcium or phosphate the first effect, which reduces the storage life of the milk, predominated at low potassium or sodium levels (0.1 *M*); whereas, the second or dilution effect, which stabilizes the milk, was more important at higher levels (0.5 and 1.0 *M*). In the absence of added calcium or phosphate, the dilution effect predominated throughout (except at 0° F. in Figure 2A). However, the ability of added potassium chloride to promote gel formation reduced its stabilizing effect, as compared to that of sodium, and the higher eutectic temperature of potassium chloride also limited the effectiveness of high concentrations at 0° F.

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TRITIUM RADIOACTIVE–CASEIN AND CHEESE RIPENING

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SUMMARY

Investigations were concerned with the preparation of casein containing radioactive hydrogen, tritium, suitable for cheese-ripening studies, the properties of the radioactive casein, and the appearance of the radioactive hydrogen of the casein in some nonprotein component of ripened cheese. The addition of tritium to casein was accomplished by the gas-exposure technique, producing a concentration of 6 mc. of tritium per gram of casein. The tritium labelling observed was that of random distribution of the isotopic hydrogen in the various amino acids of the casein. Introduction of the tritiated casein into a cheese system did not affect the ripening process. Cheese prepared with labelled casein showed the same changes in the free amino acids, rate of acid formation, and soluble nitrogen as the unlabelled cheese. An appreciable concentration of tritium was observed in two carbonyls of ripened cheese, pointing to protein as a possible source of these compounds. Tritium labelling was shown applicable in casein-degradation studies and, with certain reservations, may be extended to other compounds of cheese ripening.

The use of isotopes in dairy product chemistry has not been extensive, but an awareness of its potential exists. Application to the ripening of cheese was first pointed out in 1952 at the FAO, United Nations Cheese Conference held in Poligny, France (11). Bassett and Harper (1) at Ohio have since initiated studies employing C^{14}-labelled compounds. Tagged molecules permit tracing of the chemical changes of the active molecule in the system. In the field of animal husbandry, Black *et al.* (2) determined many of the metabolic pathways of the cow, and Glascock (6), in England, labelled dietary components with the radioactive isotope, tritium (along with C^{14} and deuterium), and injected them into goats, to show the metabolic pathways of the lactating animals.

A primary advantage of the radioisotope technique in studies of cheese ripening is the great sensitivity of the measurements obtainable. Many of the elements and compounds of interest are in the cheese in such small amounts that chemical measurements are impossible. For example, 1 mg. of cobalt per day will satisfy the requirements of a 1,000-lb. cow for this element. The chemical measurement of this amount of cobalt distributed through the tissues and excreted by such an animal is practically impossible by chemical means. However, by the use of radioactive cobalt it is a relatively simple matter to study the distribution of less than 0.1 mg. of cobalt in the cow (5).

The most logical radioactive elements to employ in labelling milk or milk constituents for cheese-ripening studies are carbon (C^{14}) and tritium (H^3), because of the abundance of carbon and hydrogen in the compounds of milk and ripened cheese.

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Presently, the only method for preparing labelled milk constituents utilizes biological processes, i.e., the introduction of a C^{14} or H^3 precursor into a lactating cow. It is difficult, however, to produce highly radioactive materials by this technique, because of the dilution of the labelled milk precursor by unlabelled precursors manufactured by the cow.

The technique described by Wilzbach (17), in which tritium is exchanged for protium, light hydrogen, H^1, is a convenient means of introducing large amounts of tritium into the milk components.

This investigation is concerned with 1) the preparation of tritiated casein suitable for cheese-ripening studies, 2) the properties of tritiated casein, and 3) the appearance of the tritium and of the tritiated casein in some nonprotein components of ripened cheese.

METHODS AND MATERIALS

Counting of tritium. A windowless gas flow counter (Tracerlab SC-18) was employed for the detection of tritium, with approximately 1% counting efficiency. For the most part, the preparations were placed on 1-in. stainless steel planchets and were either infinitely thin or infinitely thick so that no self-absorption corrections were necessary. The infinitely thick samples usually were of standard thickness, prepared on 55 mg. of No. 1 Whatman filter paper. The mass necessary for an infinitely thick tritium sample can be expressed by the formula $0.75 \times \text{range} = \text{mg/cm}^2$. This technique simplified the procedure, since counting rate depends only upon the concentration of activity in the sample and not upon the amount of sample being measured.

The paper chromatograms were assayed for radioactivity by cutting the sheets of paper into 1-in. squares and mounting each inch square on a planchet. These 1-in. squares were secured on the planchet by placing a brass washer on top of it. Each square was then placed in the windowless flow counter and the amount of radioactivity was determined.

Precautions against contamination. To avoid contamination, all working areas were covered with paper, which was regularly replaced. Areas suspected of contamination were wiped with a damp piece of filter paper, dried, and placed in the counter. Gross contamination was monitored with a Landsverk electrometer. In general, the tritiated samples were treated in the same manner as if they were microbial pathogens. There is actually very little hazard in handling tritium, because the maximum body burden is 10 μc. (15) and in routine analyses this level is never reached.

Preparation of casein. Isoelectric casein was prepared by the techniques described by Black (3), with the modification that much more care was taken to remove extraneous material from the protein. Before and after tritiation it was necessary to make certain that the labelled casein was free of the components under investigation, so they would not be incorporated into the cheese milk. The water-soluble materials were removed by reprecipitation and washing until the counts per minute per gram of casein were constant and no radioactivity was detected in the wash water. In addition, the casein was purified by repeated

extraction with petroleum ether, until no fat could be detected visually in the petroleum ether fraction.

Tritiation of casein by the Wilzbach technique. A finely powdered isoelectric casein was prepared, to obtain maximum efficiency of tritiation (15, 16). The casein powder was prepared by lyophilizing isoelectric casein and grinding it in a mortar.

Tritiation of casein was accomplished by incubating 4.6 g. of the protein with 12.8 curies of H^3 at a pressure of 0.39 atm. for 72 hr. at 27° C.[1] The tritiated casein was subjected to repeated isoelectric precipitations and lyophilizations, until constant activity was obtained in the casein, i.e., all labile tritium was removed. A concentration of 6.5 mc. of tritium per gram was attained.

Acid hydrolysis. Tritiated casein was acid-hydrolyzed by the method described by Block (4). The resultant hydrolysate was chromatographed and assayed for tritium.

Butter oil. Butter oil was prepared from sweet cream butter. Sweet butter was melted and repeatedly washed with warm distilled water and centrifuged to remove water-soluble compounds. The oil was then dried and stored at 20° F.

Starter. Cultures of *Streptococcus lactis HBN-5, Lactobacillus casei C.,* and *L. casei 7469* were used.[2] A 1:1 mixture of the two lactobacilli was employed in the samples containing *L. casei.*

Preparation of Cheddar-like cheese. A mixture containing 9.5% skimmilk solids, 3.5% purified butter oil, in a 0.1% $CaCl_2$ solution was homogenized with a hand homogenizer. Samples of 30 ml. of this mixture were used for the preparation of Cheddar cheese. Thirty milligrams of tritiated casein containing 0.19 mc. of tritium were then added and the 30 ml. of cheese milk were placed in 25- by 150-mm. Pyrex tubes. A 1.5% *S. lactis* inoculation was employed as a starter. Rennet was added at the rate of 90 ml/1,000 lb. milk 1 hr. after starter addition, and steps resembling those of Cheddar manufacturing methods were then followed. The whey was expelled by centrifuging at approximately 1,000 r.p.m. for 5 min., and the curds were then salted with 2.5% salt. A tube containing pyrogallic acid was inserted inside the 25- by 150-mm. tube and concentrated NaOH was added. The larger tube was sealed to establish anaerobic conditions. The samples were then ripened at 72° F.

Chromatographic procedures for detection of carbonyls. A 6% cheese suspension in water was placed in a Waring blendor and mixed for 1 min. The slurry was then filtered through Whatman No. 12 fluted filter paper.

Twenty-five milliliters of the filtrate was made up to a volume of 100 ml. with 95% ethyl alcohol. After the precipitate formed, the mixture was filtered through a Whatman No. 42 filter paper.

Twenty-five milliliters of the alcohol filtrate was reacted with 1 ml. of 0.1% 2,4-dinitrophenyl hydrazine at 40° F. overnight. The solution was then extracted with 50 ml. of 1:1 heptane:benzene mixture and re-extracted with another

[1] Carried out at the New England Nuclear Corporation, Boston, Massachusetts.

[2] Obtained from Dr. H. B. Naylor, Laboratory of Bacteriology, Cornell University, Ithaca, New York.

25 ml. of the benzene: heptane mixture. The total extract was evaporated on a hot plate until only 2-3 ml. remained.

The extract was chromatographed according to the directions of Heulin (7). Approximately 0.1 ml. of the solution was spotted over an area no greater than 0.25 in. in diameter, on Whatman No. 1 filter paper. The solvent used was a mixture of methyl alcohol and heptane. Twenty-five milliliters of heptane was added to 130 ml. methanol and allowed to equilibrate in a separatory funnel. The lower layer was used as the equilibration mixture. An additional 3 ml. of heptane was added to the upper layer and this mixture was used as the solvent. After the paper was placed in the chromatographic chamber, it was equilibrated for 18 hr. and the solvent was added. It required 4–5 hr. for the front to travel three-quarters of the way up the paper. The spots appeared on the paper as yellow areas.

Amino Acids. The alcohol filtrate prepared for carbonyl analysis was also used for amino acid analysis and chromatographed in two dimensions by the method described by Kosikowski (9).

Soluble nitrogen. The micro method described by Johnson (8) was used to determine the amount of alcohol-soluble nitrogen present. From the alcohol filtrate of the cheese sample, 0.3 ml. was pipetted into an 18- by 150-mm. Pyrex tube and 0.5 ml. of $4(NH_4)_2\text{-}SO_4$ containing 0.5 g. of Cu SeO_3 was added. The contents of the tubes were digested overnight in an electrically heated sand bath. After digestion, 2 ml. of water, 2 ml. of color reagent, and 3 ml. of 0.2 NaOH were added. (The color reagent contained, per liter, 4 g. of KI, 4 g. of HgI_2, and 1 g. of polyvinyl alcohol.) After standing 15 min., the tube was placed in a Bausch-Lomb photoelectric colorimeter and read at 490 mμ.

RESULTS

Purification and labelling of casein. A problem encountered in the purification of casein was the elimination of fat and other water-insoluble materials present. It was necessary to wash the casein repeatedly with fat solvents, because the residual fat which was extracted from the casein contained tritium activity. Most of the tritium in fat extracted from the casein could be removed from the fat by washing the fat with water (Table 1).

TABLE 1

Lability of H^3 in tritiated casein

Number of isoelectric precipitations after tritiation	Per cent recovery of isoelectric casein	Counts per minute per gram of casein
0	100[a]	0
1	97	4.3×10^5 [b]
2	95	4.7×10^5
3	94	4.5×10^5
6	94	4.5×10^5

[a] Untritiated casein used as 100% value.

[b] Counts were measured after an initial washing with water and one isoelectric precipitation to eliminate most labile tritium.

Labelling casein with tritium by chemical means (using the Wilzbach technique), a specific activity of 6.5 mc. per gram of casein, or 93.3 mc. per gram of hydrogen, was attained. In this chemical gas exposure technique for tritium-labelling, casein is exposed to curie amounts of tritium gas for several days. The energy released in the disintegration of the tritium and absorbed in the system provides the activation necessary to effect labelling. Since appreciable quantities of energy, 1.8×10^{19} electron volts per curie-day of tritium are involved, some radiation damage to casein occurs. Efficient purification procedures are required to remove any of the denatured protein and to obtain the tritiated casein radiochemically pure.

Milk tritiated by metabolic means apparently can not be used in cheese-ripening studies without prior separation and purification of the components. The tritium would appear where protium is present, including the water, and it would be extremely difficult to trace the breakdown products of this milk by tracer techniques. It may be possible to use metabolically labelled carbon^{14}, for exchange would not occur as freely. Using carbon^{14}-labelled casein and butterfat,[3] detection of the degradation of the components was possible, but it was difficult to trace the individual degradation compounds with the prevailing techniques and equipment. In general, the advantage of metabolic labelling is the specificity. This advantage can be achieved in certain instances with chemical labelling. For example, Pearlman (13), in his studies of progesterone metabolism, prepared progesterone tagged with tritium in the 16 position by differential catalytic hydrogenation.

Properties of tritiated casein. Repeated isoelectric precipitation of the casein after tritiation did not result in any significant loss of casein (Table 1). The results indicate that no significant denaturation took place during the tritiation process. Total activity of the precipitates is included in Table 1, to point out the absence of water-exchangeable or water-soluble tritium.

The tritiated casein and 6 *N* hydrochloric acid were placed in a sealed test tube in an autoclave (121° C. at 15-lb. pressure) and were acid-hydrolyzed for 18 hr. The hydrolysate was dried in vacuo and the radioactivity determined. Eighty per cent of the original tritium activity was recovered in the dry material. The loss in activity can probably be attributed to (a) introduction of protium during hydrolysis of the peptide bonds of the casein, (b) the loss of tritium during the destruction of cysteine and tryptophane, and (c) mechanical losses. The bulk of the radioactivity, however, was recovered in the amino acids after acid hydrolysis.

Counts for individual amino acids obtained by chromatography of acid-hydrolyzed casein demonstrated that the tritium was randomly distributed in the various amino acid molecules (Figure 1). The paper chromatogram was cut into 1-in squares and each square was counted for specific activity of the amino acids (Table 2). With the possible exception of the aromatic amino acids, gen-

[3] Supplied by M. Kleiber and J. Luick, College of Agriculture, University of California, Davis, 1957.

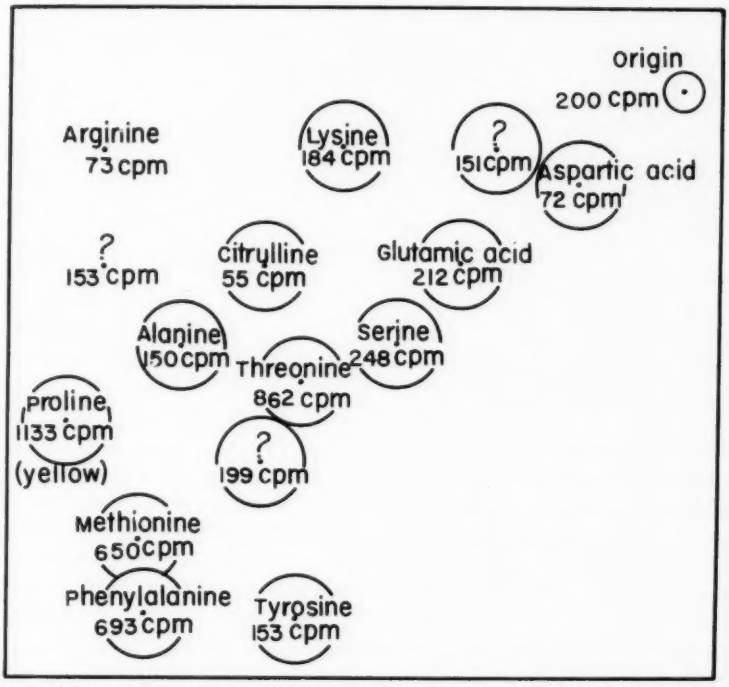


FIG. 1. A paper chromatogram showing location of amino acids from hydrolyzed tritiated casein and the counts per minute at each area.

C—colored blue areas.
•—Detected radioactivity.
?—Amino acid not identified.
c.p.m.—counts per minute.

erally, the label was randomly distributed in the casein molecule. Wilzbach (17) has also reported that tritium-labelling shows up in protein at all positions, but not uniformly.

Cheese ripening. Cheese prepared with tritiated casein showed the same ripening changes in pH, soluble nitrogen, and in the free amino acid spectra as unlabelled cheese. The radioactivity in the cheese apparently did not have a measurable biological effect on cheese-ripening and there was no isotope discrimination as a consequence of the increase in molecular weight, for the products had the same concentration of tritium as the parent molecule.

At various time intervals during the progression of cheese ripening, samples of cheese were homogenized and filtered. Portions of the filtrates were used for radioactivity determinations. Alcohol was added to the 25 ml. of the filtrate to 75% by volume, to precipitate peptides. The precipitate was removed by filtration and a portion of the alcohol-soluble material was removed for counting. Carbonyl compounds were extracted from the alcohol-soluble materials and

TABLE 2
Tritium activity in amino acids of casein

Amino Acids	Amino acid composition of casein calculated to 16 g. of nitrogen[a]	Counts per minute[b]
Valine	7.4	506
Proline	12.3	1,153
Phenylalanine	5.8	693
Tyrosine	6.3	153
Glutamic Acid	23.6	212
Aspartic Acid	6.5	72
Serine	6.3	360
Arginine	4.2	73
Lysine	8.2	84
Methionine	3.3	650
Threonine	4.5	862

[a] Values obtained from Block and Weiss, *Amino Acid Handbook*, Charles C Thomas, Publisher, Bannerstone House, Springfield, Ill.
[b] Data from present investigation.

analyzed as described under the section on Methods. The carbonyl phenylhydrazones were counted after cutting out the portions of the paper chromatograms containing the phenylhydrazones. Theoretically, the specific activity of the cheese component formed only from casein is of the same order of magnitude as that of the casein. A large decrease in the specific activity indicates that milk constituents other than casein may be contributing to the formation of cheese components, such as carbonyls. The specific activity of the total water-soluble and alcohol-soluble components of a ripened cheese varied with ripening time, and indicated the degree to which the casein contributes to the fraction at the various periods of ripening (Table 3).

Acetone and C7 carbonyl detected visually on paper chromatograms contained practically no radioactivity and, therefore, could hardly have arisen apppreciably from casein. Significant activity, however, was found in two different spots on the chromatogram (Table 4). These compounds have not been completely identified, but the position of one corresponds to pyruvic acid or other slow-moving

TABLE 3
Comparison of specific activity of total water-soluble and alcohol-soluble material from ripened cheese

Time (days)	Total H_2O-soluble	Alcohol-soluble material
Cheese made with *S. lactis* starter	Counts per minute	Counts per minute
.25	3.1×10^4	2.2×10^4
7	4.4×10^4	1.9×10^4
17	[a]	1.0×10^4
21	[a]	2.3×10^4
Cheese made with *S. lactis* + 10 *L. casei* cells/ml milk		
.25	2.6×10^3	1.6×10^3
7	3.0×10^3	1.0×10^3
14	5.3×10^4	1.6×10^4
21	5.9×10^4	5.8×10^3

[a] Samples not analyzed.

TABLE 4
Appearance of labelled carbonyls in ripening cheese made with *S. lactis* starter

Time	Slow-moving carbonyl (Reference 1)	Faster-moving carbonyl (Reference 3)
(days)	*(counts per minute)*	*(counts per minute)*
.25	1	70
7	14	98
14	63	4
21	96	11

carbonyls; whereas, the position of the other spot corresponds to that of diacetyl, formaldehyde, or acrolein.

Throughout the ripening, radioactivity in very low amounts was observed in other carbonyls than the two mentioned. The activity in these carbonyls, although not statistically significant, indicates the degradation of casein into other carbonyls as well.

DISCUSSION

The technique of tritiation described by Wilzbach (17) permits tritium labelling of compounds which hitherto could only be tagged via metabolic routes. It is a relatively simple and nonspecific means of labelling milk constituents. The technique is essentially an exchange reaction of tritium for protium. The present study shows that casein, tritiated by the Wilzbach technique, is useful for cheese-ripening studies.

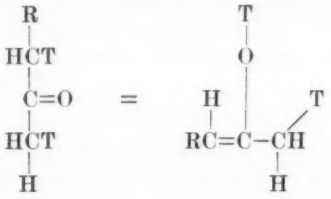
The usual techniques for incorporating tritium into materials are similar to those employed for deuterium. The labelling can be obtained by reduction, exchange, hydrolysis, or via intermediates. Reduction with tritium or metal hydrides ($NaBH^3_4$ $LiAlH^3_4$) at double bond or carbonyl sites is the most direct method leading to high specific activities. The recently developed Wilzbach technique is essentially an exchange reaction where the organic compound is allowed to stand at room temperature with subatmospheric (0.51 atm.) pressures of tritium, yielding a product of high specific activity.

Mild conditions employed during the Wilzbach tritiation decrease the likelihood of gross chemical and physical changes in the material. Steinberg (14), for example, reports that only 10% denaturation of crystalline lysozyme occurred during an exposure of 72 hr. to 1.8 curies of tritium.

The large mass difference between tritium, deuterium, and protium leads to many distinct differences in chemical and physical properties of the three isotopes. In most tracer research involving hydrogen, the major interest is in labilization of carbon-hydrogen bonds. The carbon-protium bond is somewhat looser than the carbon-tritium bond. The reason for this is that as the effective mass of the isotope increases, the "zero-point" enery is lowered. Before the isotope can be split away from the carbon and react, it must be given activation energy. The lower the zero-point energy, the more activation energy is required. Hence, tritium is more difficult to activate than protium. Since hydrogen-transporting enzymes are extremely sensitive to small gradations in activation of C-H bonds, there exists a possibility that isotope discrimination reactions can occur

in enzyme-catalyzed reactions, which can invalidate the use of tritium as a tracer for hydrogen.

Stable linkages between tritium and carbon permit the use of tritium as an auxiliary tracer for carbon. Tritium can not be stably linked to oxygen or nitrogen atoms as in $-NH_2$, $-OH$ because, in aqueous media, dissociation occurs and equilibration of tritium results between the labelled linkage and water. Compounds tagged with tritium in this way (RCH3COOT), (RCHTNHTCOOT), etc., lose tritium by exchange, and are generally of no value in tracer experiments. Less labile linkages occur when the carbon-tritium bond is attached in an adjacent position to a carbonyl group. Under these circumstances, an enolization equilibrium results and formation of a labile oxygen-tritium linkage develops, i.e.,



The strength of carbon-tritium bonds allows the preparation of stable labelled compounds if care is taken to remove unstable bonds such as oxygen-tritium and nitrogen-tritium.

In measurements of tritium, it is important to recognize the possibility of tritium exchange and the necessity of washing the samples to constant activity.

Tritium has become a useful tool in biological chemistry because it is the only radioactive form of hydrogen, the most common element in organic compounds. Tritium has a low maximum beta energy, 0.0189 Mev, compared to, for instance, C^{14}, which has a maximum of 0.155 Mev. However, because of the low beta energy and the low penetrating power of H^3, it is difficult to count, and specialized techniques have to be used, such as gas counting in ionization chambers, scintillation counting, or windowless gas-flow counting. For soft-beta counting, the gas-flow counter in the Geiger regions is used. Essentially, this is a shielded counter into which solid samples are inserted directly and through which there is a gas flow to prevent air contamination. The thinnest mica window counters absorb over 50% of the beta particles from S^{35} or C^{14} and do not permit passage of H^3 particles. The windowless counter is several times more sensitive for C^{14} and S^{35} than is the thin window-type counter. The increased sensitivity results from the fact that there is no loss due to air and window absorption; also, the geometry factor is high. Extreme care must be taken in handling the windowless counter to prevent contamination, for the sample is actually introduced into the Geiger tube. The sample must be thoroughly dry and without pockets of entrapped air or solvent which may poison the counting atmosphere.

Emphasis in the present study was on the formation of tritiated nonprotein small molecules from tritiated casein during cheese maturation. By measuring the amount of a tritiated small molecule versus its absolute amount, rough esti-

mates of the contribution of nonprotein components to the formation of a particular group of compounds such as carbonyl compounds were possible. The investigation of carbonyls was stressed because of their production from proteins by lactic acid organisms (10) and their presence in cheese as possible flavor constituents (12).

The appearance of the tritium in these carbonyls is indicative of degradation of the protein to amino acids and then removal of the amino group of the amino acid. The mechanism for the removal of the amino group may be either oxidative deamination or transamination. Although the tritium appears in significant amounts only in some carbonyls, this does not eliminate the possibility that the other carbonyls are being formed, to some degree, at the same time, in the same manner. These investigations showed only those compounds which were predominantly arising by this route.

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INTERRELATIONSHIPS BETWEEN pH, POPULATIONS OF *PROPIONIBACTERIUM SHERMANII*, LEVELS OF FREE FATTY ACIDS, AND THE FLAVOR RATINGS OF SWISS CHEESES

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SUMMARY

Fifteen experimental cheeses were examined periodically during 1 yr. The following variations in the normal cheeses were found: pH off the press (experimentally induced variation), 4.95–5.37; maximum numbers of *Propionibacterium shermanii*, 145 million/g–2,100 million/g; maximum levels of acids—acetic, 38–59; propionic, 44–104; butyric, 6–40; higher, 22–48 μM/g. With added sodium propionate, pH and numbers of *P. shermanii* increased but production of propionic acid decreased. Five commercial cheeses, examined for flavor and fatty acid levels, typically had higher levels of acetic acid than the experimental cheeses.

Significant relationships were found between pH off the press and growth of *P. shermanii* and also between the maximum levels of propionic acid and both the maximum numbers of *P. shermanii* and the *P. shermanii* numbers-time integrals. All relationships involving the "sweet" flavor component are without significance. With one exception, all relationships involving the "nutty" flavor component are either of doubtful or of no significance. The relationship between nutty flavor and propionic acid levels may be significant.

Propionic acid is a component of the flavor complex, but other compounds appear to be responsible for both the nutty and the sweet characteristics of typical Swiss cheese flavor.

Positive correlations between fatty acid levels and flavor of Swiss cheeses have been reported by several investigators (1, 2, 7, 11, 12). Propionic acid has been considered the most important of the volatile acids in contributing desirable characteristics to Swiss cheese flavor. Babel and Hammer (2) tested 18 strains of propionic acid bacteria and found that, with certain strains, the amount of culture used was correlated positively with the intensity of sweet flavor and the level of volatile acids in the aged cheese. Demeter (3) considered that the propionic acid organisms were very important in the development of flavor in Swiss cheese but that, nevertheless, propionic acid was not the substance responsible for the flavor. Krett *et al.* (10) reported the levels of various fatty acids, but no other values for comparison, during the aging of a typical Swiss cheese.

Frazier *et al.* (5), from a study of over 800 cheeses made under factory conditions, found that the group having a pH of 5.0–5.2 off the press contained the highest percentage of top-quality cheeses. The limit of 5.2 might be exceeded, if the milk were of high quality. Sherman (14) reported that propionibacteria were responsible for the development of characteristic flavor. In a study of cultures, Tittsler (15) found that the optimum pH for growth of *Propionibacterium shermanii* was from 6.0 to 7.0. With increasing distance from the optimum range the initiation of growth was delayed and the rate of growth reduced. If growth occurred, however, the maximum populations reached were the same, regardless of the initial pH.

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These various studies have been sufficiently encouraging regarding a correlation between the flavor of Swiss cheese and factors which are susceptible to control in its manufacture, to prompt the present study of the interrelationships between pH, populations of *P. shermanii*, fatty acid levels, and flavor.

EXPERIMENTAL PROCEDURE

Fifteen experimental cheeses, approximately 60 lb. each, were manufactured by a standard procedure (13), except as noted. The starters used were *Streptococcus thermophilus* (S), *Lactobacillus bulgaricus* (Ga), and *P. shermanii* (62). The cheeses were held in the warm room for 4 wk. at a temperature of 72° F. The temperature of the cold room was 50° F. Variations in the pH of the cheeses off the press were experimentally induced by varying the quantity of *S. thermophilus* or *L. bulgaricus*, or by replacing varying amounts of whey with water. A mixture of 1 lb. of propionic acid and 5.9 lb. of sodium propionate was added to the 700 lb. of milk used for making one cheese for which, otherwise, the manufacturing process was normal. Another cheese was made with the addition of the same amounts of propionic acid and sodium propionate as above, but with the omission of the *P. shermanii* starter.

The cheeses were aged for about 1 yr. and examined over this period for flavor, pH, numbers of *P. shermanii*, enterococci, and spore-formers, and for levels of acetic acid, propionic acid, and higher acids as a group. Samples were taken of cheeses off the press and subsequently at intervals varying from 2 wk. for young cheeses to 8 wk. for those 6 mo. or older. Also, pH measurements were made at dipping and at 3 hr. on the press. In addition to the experimental cheeses, four imported cheeses and one domestic commercial cheese, selected for quality, were examined for flavor and fatty acid levels.

Flavor ratings were determined by a panel of experienced judges. Fatty acids were determined by the method of Harper (6), with minor modifications. Ten-milliliter samples of eluate were collected, and meta cresol purple was used as the indicator. In nonaqueous solvents this indicator, in contrast to phenol red, has a pronounced color change near the equivalence point. The titrimetric results are expressed as micromoles per gram of cheese.

The numbers of bacteria were determined in the following manner. They were grown in a medium consisting of 0.5% each of tryptose, tryptone, yeast extract, and sodium lactate and 1.5% agar. Two series of lactate agar plates, with agar seals, were poured for each cheese sample; one was incubated at 45° C. for 3 days and the other at 30° C. for 10 days. This medium, at 30°, supports the growth of propionibacteria but none of the other starter types. During previous research in this laboratory on Swiss cheese it was established that growth on the 45° C. plates is restricted primarily to members of the enterococcus group. Spore-formers, if present, also grow on these plates but are differentiated from the enterococci by colony type. Colonies of propionibacteria begin to appear on the 30° C. plates after 3 days of incubation. They were differentiated from the enterococci by colony type and counted after 10 days of incubation.

RESULTS AND DISCUSSION

Development of P. shermanii. The cheeses were put into the warm room approximately 2 wk. after manufacture. At that time, as expected, none of them contained a large number of *P. shermanii.* The subsequent development varied considerably in the different cheeses. After 2 wk. in the warm room, the populations had increased three to 980 times the corresponding numbers off the press. In 40% of the normal cheeses the maximum populations were reached 6 wk. after manufacture, which coincided with the time of their removal from the warm room. The time at which the maximum populations were reached in the other cheeses varied from 2 to 7 wk. after removal from the warm room. Details of these results are given in Table 1.

TABLE 1

Relation between growth of *Propionibacterium shermanii* and pH off the press

Cheese No.	pH	Population ratio of *P. shermanii*:[b] (No. at 4 wk.)/(No. off the press)	Age of cheese at time of maximum numbers
			(wk.)
1	4.95	.3	13
4	4.97	12	10
2	5.00	14	10
5	5.10	10	13
12	5.15	24	6
3	5.20	65	8
14	5.22	60	6
11	5.25	267	6
10	5.35	425	6
6	5.37	980	8
15[a]	5.82	120	8

[a] Sodium propionate was added to the cheese milk.
[b] Represents the number of times the population of *P. shermanii* increased in 4 wk. from the time the cheese was removed from the hoop.

It was found that not only the maximum population of *P. shermanii* but also the period of time during which the population was maintained near the maximum varied considerably from cheese to cheese. In Figure 1, Cheeses No. 6 and No. 11 represent the extremes of the normal cheeses in this respect.

The effect of pH on the development of *P. shermanii* can be considered with respect both to its effect on the maximum populations reached and on the rate of growth when the numbers are appreciably below the maximum. With respect to the latter, it was found, Table 1, that the growth of *P. shermanii* increased directly with an increase in pH of the cheese off the press. The relationship between pH and the growth of *P. shermanii* as represented by the ratio, (numbers of *P. shermanii* at 4 wk.)/(numbers of *P. shermanii* off the press), was closer than any other relationship studied in this investigation. As the initial pH's are all below 6, an increase in pH brings this value closer to the optimum range reported by Tittsler (15). The effect of pH on the early growth of *P. shermanii* in cheese is, therefore, similar to that found in cultures (4, 15).

The maximum population of *P. shermanii* varied considerably from cheese to cheese and, as seen in Figure 4, showed a general positive correlation with pH

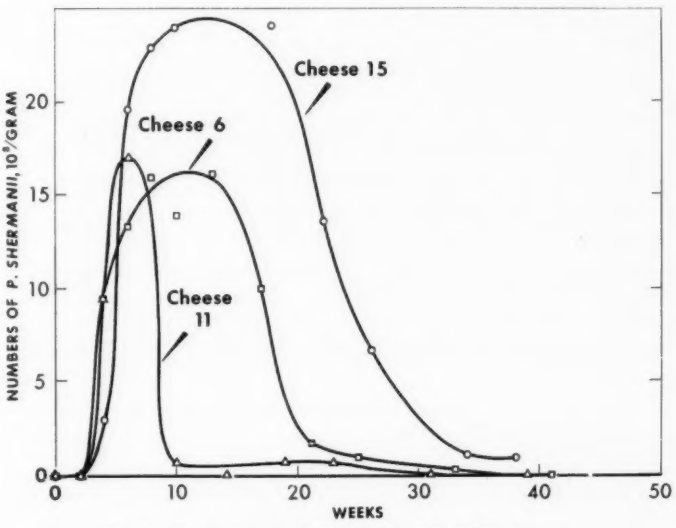

FIG. 1. Development of *P. shermanii* in Swiss cheeses.

off the press. This is in contrast to the behavior of this organism in cultures, as Tittsler found that if *P. shermanii* grew at all the ultimate maximum numbers were the same regardless of initial pH (15).

The correlation between pH and the time needed to reach the maximum population of *P. shermanii* is not as close as that with early growth. This is evident from the data in Column 4 of Table 1. This can be considered to be due to a

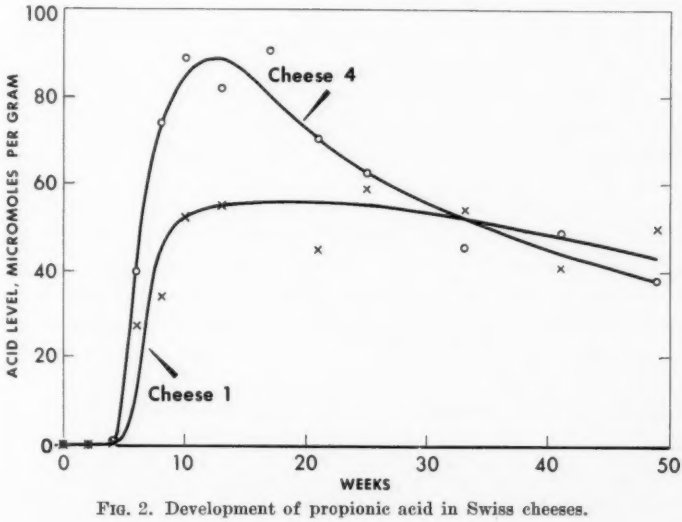

FIG. 2. Development of propionic acid in Swiss cheeses.

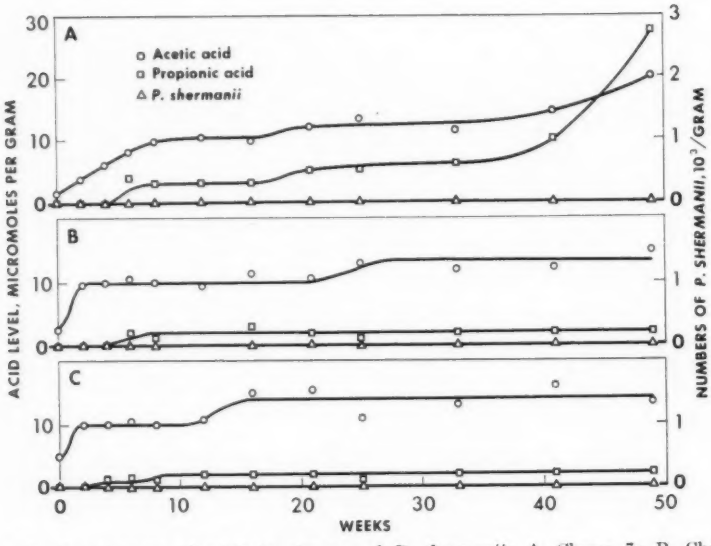

FIG. 3. Development of acids in absence of *P. shermanii*: A, Cheese 7; B, Cheese 9; C, Cheese 8.

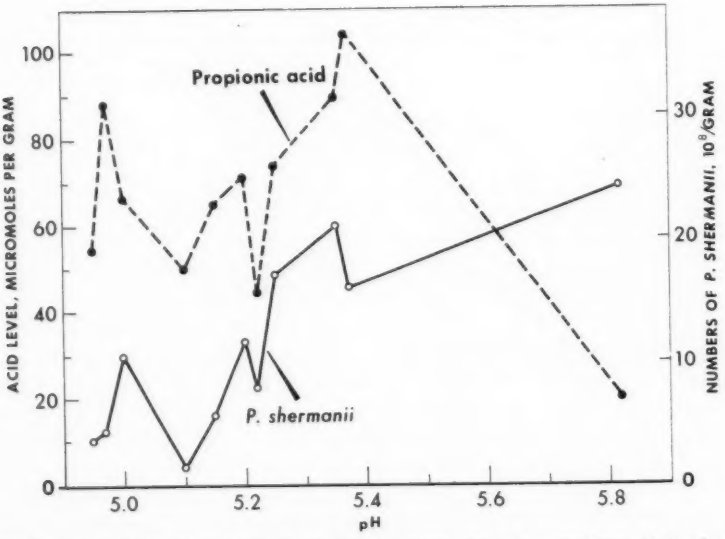

FIG. 4. Interrelationships between pH off the press, maximum numbers of *P. shermanii*, and maximum levels of propionic acid in Swiss cheeses.

modification of the relationship between pH and early growth by the inevitable narrowing of the differences between the population increases as they approach 0 at the maxima and also to the differences in population levels in the various cheeses both at off the press and at their maxima. Cheese No. 6, for instance, with an initial pH of 5.37, reached its maximum population at 8 wk., and Cheese No. 14, initial pH of 5.22, at 6 wk. During these periods, however, the numbers of *P. shermanii* in the former cheese increased more than 16 times as much as in the latter.

Cheese 5, which shows a development of *P. shermanii* somewhat out of sequence with the other cheeses, was also atypical, in that the ratio of acetic acid to propionic acid was exceptionally high. Three of the cheeses, No. 7, 8, and 9, are not included in Table 1. *P. shermanii* could not be detected in these cheeses. However, one of these cheeses, Figure 3, showed the production of considerable propionic acid.

Cheese No. 15, shown in Figure 1, was abnormal in that it was made from milk to which had been added approximately 1% of a mixture of sodium propionate and propionic acid. This mixture reduced the pH of the milk to 5.90. The pH measurements on this cheese at dipping, at 3 hr. on the press and off the press were, respectively, 5.88, 5.88, and 5.82. This seems to indicate that the *S. thermophilus* and *L. bulgaricus* starters were inactivated to such an extent that they did not convert any appreciable amount of lactose to lactic acid. *P. shermanii*, which survived in numbers comparable to those in the normal cheeses, then developed in a lactose rather than in a lactate medium. It can be seen in Table 1 that the growth of *P. shermanii* in this cheese was slower than would be expected from the pH of the cheese. This parallels the finding of El-Hagarawy *et al.* (4), who reported that in cultures this organism grows more slowly in a lactose than in a lactate medium. The maximum number of *P. shermanii* reached in this cheese, 2.4×10^9 per gram, was, however, greater than that of any other cheese. Similarly, El-Hagarawy *et al.* (4) had found that with cultures the slower growth in a lactose medium did not prevent an ultimate population as large as in a lactate medium. The *P. shermanii* in this cheese also had by far the largest numbers-time integral, i.e., the area under the numbers-time curve. However, the production of propionic acid, discussed later, was abnormally low.

Development of other organisms. Spore-formers were generally absent or, if present, rapidly declined in numbers. The data in Table 2 indicate that the non-starter bacteria capable of growth under the plating conditions employed were chiefly enterococci. The likelihood of these organisms being present in large numbers was found to increase both with increasing pH off the press and in the absence of propionibacteria.

Development of fatty acids. The development of propionic acid is illustrated in Figure 2. Generally, the maximum level of propionic acid was found at the same time, or the time of the subsequent analysis, as was the maximum population of *P. shermanii*. In most of the cheeses there appeared to be some loss of propionic acid after the maximum level had been reached. This varied from the extreme

TABLE 2
Mean populations of nonstarter bacteria

Cheese No.	pH off the press	Enterococci[a]	Total bacteria[a, b]
		(thousands per gram of cheese)	
1	4.95	0.4	0.5
4	4.97	0.4	1.1
2	5.00	0.3	0.7
5	5.10	1.1	1.6
8[c]	5.13	1,280	1,620
9[c]	5.13	5,600	7,000
12	5.15	0.1	1.7
3	5.20	0.6	1.8
14	5.22	990	1,150
11	5.25	100	128
7[c]	5.33	202,000	230,000
10	5.35	3.2	5.0
6	5.37	112,000	130,000
13[d]	5.80	730	990
15[e]	5.82	850	940

[a] Mean values of 11 to 13 determinations made over entire ripening period.
[b] As found on lactate agar plate incubated at 30° C. for three days.
[c] No propionibacteria present.
[d] *P. shermanii* omitted; propionate added.
[e] Propionate added.

in Cheese No. 4 to the more typically moderate loss in Cheese No. 1 (both shown in Figure 2) to almost nothing. The loss for each cheese can be determined from the data in Table 4. The significance of curves such as those in Figure 2, in attempting to correlate flavor with propionic acid levels, is discussed later. The cause of such a high loss of propionic acid as that found in Cheese No. 4 is not known, but in this particular cheese it was associated with an extremely poor texture which was cabbagy, with dry, slit-like eyes.

The three parts of Figure 3 pertain to the three cheeses of one day's manufacture. The complete absence of detectable organisms of the genus *Propionibacterium* in each of the three cheeses would appear to be explained most readily by assuming an inadvertent omission of the *P. shermanii* starter. The small level of propionic acid in Figures 3B and 3C is within the experimental error of its determination. The significant level of this acid, shown in Figure 3A, would appear to be an unusual instance of the formation of propionic acid in Swiss cheese by organisms other than those of the genus *Propionibacterium*. So far as the authors are aware, this has not been previously reported with substantiation.

The ratios of propionic to acetic acid found in the various cheeses are given in Table 3. Johns (8), working with cultures of *P. shermanii*, found that with increased carbon dioxide tension in the medium the ratio varied from 1.76 to 3.56 in glucose media but from only 1.90 to 2.14 in lactate media.

At 2 wk. after manufacture, before the development of appreciable propionic acid, acetic acid was invariably found to be present in the cheeses. Figure 3 shows that acetic acid, in comparable concentrations, was produced also in those cheeses in which *P. shermanii* could not be detected. It would thus appear that the acetic acid levels found in Swiss cheese result not only from the activity of *P. shermanii* but from that of other organisms as well.

TABLE 3
Ratios of propionic acid to acetic acid in Swiss cheese

Cheese No.	Ratio as found in cheese	Ratio after correction for initial production of acetic acid
1	1.5	2.4
2	1.4	1.8
3	1.3	1.6
4	1.6	2.2
5	1.1	1.7
6	2.0	2.1
7[a]	1.5	2.0
8[a]	0.1	....
9[a]	0.2	....
10	2.1	2.2
11	1.6	2.0
12	1.5	2.0
13[b]	0.0	....
14	1.0	1.6
15[c]	1.3	1.7
16[d]	1.0	....
17[d]	0.9	....
18[d]	1.0	....
19[d]	0.8	....
20[e]	0.8	....

[a] *P. shermanii* could not be found in this cheese.
[b] Sodium propionate was added to the cheese milk—*P. shermanii* was omitted.
[c] Sodium propionate was added to the cheese milk.
[d] Imported commercial cheese.
[e] Domestic commercial cheese.

TABLE 4
Free fatty acids and flavor of Swiss cheeses

Cheese No.	Age	Propionic	Acetic	Butyric	Higher	Sweet	Nutty	Off-flavor
	(wk.)	*(μM per gram)*						
5	49	29 (50)[a]	34	6	18	Medium	Absent	Absent
4	49	38 (89)	35	4	16	Slight	Absent	Absent
1	49	43 (55)	35	7	24	Medium	Absent	Absent
14	24	44 (44)	40	34	24	Slight	Absent	Malty and dirty
3	49	45 (72)	33	6	31	Slight	Absent	Absent
20	....	48	58	6	35	Slight	Slight	Acid
17[b]	....	51	59	3	17	Medium	Medium	Absent
2	49	53 (67)	37	4	24	Intense	Absent	Absent
19	....	57	72	3	17	Slight	Absent	Absent
12[b]	39	64 (66)	42	5	19	Medium	Medium	Absent
16	....	66	68	2	16	Absent	Absent	Absent
11[b]	39	72 (75)	45	5	33	Medium	Medium	Absent
18	....	77	75	3	13	Medium	Medium	Dirty
6	39	89 (104)	44	11	32	.....	.....	Rotten
10	39	90 (90)	43	6	44	Medium	Slight	Absent

[a] Parenthetical values are maxima. All other data pertain to samples taken at time shown in Column 2.
[b] Grade A cheese.

Column 2 of Table 3 lists the (propionic acid)/(acetic acid) ratios as actually found in the cheeses. Column 3 of this table lists these ratios after they have been corrected by subtracting the acetic acid level at 2 wk. from the maximum level of acetic acid reached at a later date. This is a reasonable way to account for the acetic acid produced by organisms other than *P. shermanii*. Even though such a correction must be rather inaccurate, it is clear that the corrected values are much closer than the uncorrected to the theoretical value of 2.0.

The commercial cheeses, both domestic and imported, were typified by high levels of acetic acid with the (propionic acid)/(acetic acid) ratios varying from 1.0 to 0.8. In addition to the carbon dioxide tension, Johns (8) has listed the initial and final pH of the medium, the strain of *Propionibacterium*, and the nature of the substrate as factors which would influence the (propionic acid)/(acetic acid) ratio. Information is not available as to whether the comparatively high levels of acetic acid in the commercial cheeses arose from the influence of any of these factors or were due entirely to the activity of organisms other than those of the genus *Propionibacterium*.

The levels of butyric acid and of the higher acids, at the time of the final examination of the cheeses, are given in Table 4. In general, the final levels of these acids are nearly the same as their maximum levels. The two cheeses containing added sodium propionate, not listed in this table, had the abnormally high butyric acid levels of 29 and 51 $\mu M/g$.

Flavor. It can be seen from the comments in Table 4 that the most frequent flavor defect of the cheeses was a lack of the quality described as nutty. Of ten normal experimental cheeses, this quality was absent in six, present in three, and unjudgable in one because of a pronounced off-flavor. In two of the five samples of commercial cheeses, the nutty quality was completely lacking. The sweet quality of Swiss cheeses flavor, in contrast, was completely lacking in only one of 14 cheeses. Off-flavors were found in four of the 15 cheeses.

Interrelationships. The relationship between the development of *P. shermanii* and pH has already been discussed. The interrelationships between these values and maximum levels of propionic acid can be seen in Figure 4. The production of propionic acid was just about as closely related to the *P. shermanii* numbers-time integrals as to the population maxima (Table 5).

Babel and Hammer (1, 2) thought that propionates in Swiss cheese were sweet and contributed directly to the intensity of the sweet flavor. Pette (12) also thought that propionates were directly responsible for sweetness in Swiss cheese. Krett and Stine (11) and Hintz *et al.* (7) found a positive correlation between the levels of propionates in Swiss cheeses and the intensity of characteristic flavor. These investigators, however, were noncommittal as to whether the propionates contributed directly to the characteristic flavor or whether they were correlated with the levels of some other substance or substances which were the flavor contributors.

If the former were true, the flavor ratings of a series of cheeses should show a positive correlation with the levels of propionic acid in the cheeses at the time of grading. In the latter case, since it is unlikely that the levels of the flavor

contributor would be affected by aging in exactly the same way as would those of propionic acid, observed correlations between flavor ratings and propionic acid levels would be affected by the age of the cheeses. The correlations would be affected adversely by the extent to which the cheeses under comparison differed in the percentage loss of propionic acid with aging, and might be closest at the time of maximum levels of propionic acid.

Aqueous solutions of propionic acid and propionate ion (as sodium propionate) in proportions and total concentrations found in Grade A cheeses were tested for flavor organoleptically. The solutions were devoid of either a sweet or a nutty characteristic, but were salty and had a quality which could be described as rich and which was reminiscent of Cheddar rather than of Swiss cheese. However, the flavor of propionic acid might be modified in the complex environment of cheese. Therefore, its flavor in water is evidence, but not proof, that it is not the contributor of either the sweet or the nutty characteristic of Swiss cheese. The data in Table 4 also indicate that the sweet characteristic, at least, is unrelated to the propionic acid level. Propionic acid appears to be neither the sweet flavor component nor an indicator of its presence. The relationship between the nutty characteristic and propionic acid is discussed below.

Hintz *et al.* (7) reported that a minimum propionic acid concentration of 5.0 mg. per gram of cheese was necessary before the cheese possessed a satisfactory Swiss cheese flavor. This is equivalent to 67 $\mu M/g$. The levels of propionic acid found in the two experimental Grade A cheeses are very close to this value. However, the concentration of 51 μM of propionic acid per gram of cheese found in the imported Grade A cheese would indicate that the minimum value reported in the literature can not be considered to be a general requirement for Swiss cheese of satisfactory flavor.

Propionic acid is flavorful and, therefore, is a component of the flavor complex. It would appear, however, that other compounds are responsible for both the nutty and the sweet characteristics of typical Swiss cheese flavor. Taken as a whole, the analyses indicate that it is much more difficult to manufacture Swiss cheese with a satisfactory level of characteristic flavor than with a satisfactory level of propionic acid.

Numerical evaluation of the interrelationships has been made by the methods described by Kendall (9). The results are given in Table 5. The correlation coefficient, τ, can vary from +1 through 0 to −1. This is true whether the qualities whose relationship is being tested are dependent or independent. If independent, however, the value of τ will most probably be near to 0 and least probably near to ± 1. The probability, P, that a value of τ can have been obtained from a relationship between independent qualities can, therefore, be used as a test of the significance of τ. The lower the value of P, which is variable between 0 and 1, the greater is the significance of τ and the greater the probability that the relationship is between dependent qualities. The choice of an exact value of P for considering τ significant must be arbitrary. A value of 0.05 or less, however, is often considered sufficiently low to justify an assumption that a relationship is significant.

TABLE 5

Various relationships in Swiss cheeses

Relationship	r	P
$\left[\frac{\text{No. of } \textit{Propionibacterium shermanii} \text{ at 4 wk.}}{\text{No. of } \textit{P. shermanii} \text{ off the press}}\right]$ and pH off the press	.87	$.0^21$
P. shermanii maxima and pH off the press	.64	$.0^29$
P. shermanii maxima and propionic acid maxima	.56	.03
P. shermanii no.–time integral and propionic acid maxima	.51	.05
Propionic acid maxima and pH off the press	.38	.16
Sweet flavor and propionic acid content	.12	.59
Nutty flavor and propionic acid content	.47	.05
Sweet flavor and propionic acid maxima	.04	1.00
Nutty flavor and propionic acid maxima	.30	.38
Sweet flavor and *P. shermanii* maxima	.04	1.00
Nutty flavor and *P. shermanii* maxima	.37	.26
Sweet flavor and pH off the press	−.10	.81
Nutty flavor and pH off the press	.45	.17
Sweet flavor and *P. shermanii* no.–time integral	.10	.81
Nutty flavor and *P. shermanii* no.–time integral	.22	.53

Using this test of significance, the data in Table 5 indicate the following: The relationships between pH off the press and both the growth and maximum numbers of *P. shermanii* are highly significant. The relationships between the maximum levels of propionic acid and both the maximum numbers of *P. shermanii* and the *P. shermanii* numbers-time integrals are significant. The correlation between maximum levels of propionic acid and pH off the press is not high enough to exclude the possibility that the relationship could have arisen by chance.

There is no significance to any of the relationships involving the sweet component of Swiss cheese flavor. With one exception, all the relationships involving the nutty flavor component have correlation coefficients which are too low to justify the assumption of significance. The exception is the relationship between the nutty flavor component and the propionic acid level at the time of judging. The probability of only 0.05 that the observed relationship could have occurred by chance is sufficiently low to warrant the assumption of significance. However, organoleptic tests indicated that it is improbable that propionic acid, itself, is the nutty flavor component. Also, if the acid acted as an indicator of nutty flavor, one would expect a closer correlation than was found between the maximum level of the acid and nutty flavor. Thus, it is felt that this relationship is best described by saying that it may be significant.

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EFFECT OF SKIMMILK HEAT TREATMENTS ON COTTAGE CHEESE MANUFACTURE [1]

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SUMMARY

Calcium chloride, anhydrous, when used at the rate of 0.02% of the skimmilk, was not beneficial in the manufacture of cottage cheese from pasteurized skimmilk, from skimmilk heated to 175° F. for 30 min., from "low-heat" nonfat dry milk (NDM), or from "bakery-type" NDM.

Heat treatments of concentrated skimmilk, such as might occur in condensing or in drying, damage the cheese-making properties of the milk after reconstitution. However, such heat treatments did not induce measurable changes in solubility of whey proteins as measured by the Harland-Ashworth method, A.D.S.A. curd tension test, or solubility index, but they did decrease the strength of casein coagulum as measured by the rennet and the rennet-calcium tests for curd strength.

Cottage cheese of good commercial quality was manufactured from fluid skimmilk which had been heated to 175° F. for 30 min. to denature the whey proteins; the cheese was made by using up to 20 ml. rennet per 1,000 lb. of skimmilk and cutting the curd at the A-C end-point. Gains in yields of curd by increased recovery of milk solids approximated 10%. Time from adding starter to cutting was decreased. When normal making procedures were followed after cutting, the finished cottage cheese was criticized more often than the low-heat controls for slight mealiness, slight lack of uniformity of particle size, and pH that was considered to be too high for best keeping quality.

Many investigators, in studying the quality of the cottage cheese made from reconstituted nonfat dry milk (NDM), have stressed the significance of heat treatments of skimmilk, before and during processing of NDM. Morris *et al.* (13) found that heat treatments of skimmilk producing greater than 10% denatured whey proteins resulted in cottage cheese of inferior quality (7). Stone *et al.* (22) observed that preheat treatments of the skimmilk at 185° F. for 10 min. produced NDM with poor curd-forming properties.

Rudnick (17) subjected portions of identical skimmilk to different heat treatments and made them into cottage cheese, using no rennet. He found that when the curd was cut at the same pH, the presence of denatured whey proteins in the skimmilk reduced the whey-expelling powers of the curd and produced fragile curd. He also noted that curd made from reconstituted nonfat dry milk, which had been manufactured by condensing and drying on experimental equipment, was more fragile than curd made from the normal skimmilk control. He observed no marked changes in the moisture-expelling properties of the curd, provided that the level of whey protein denaturation remained unchanged. The fragility defect, however, was greatly accentuated by superheating the concentrated skimmilk or by raising the temperatures to which the concentrate was exposed during drying.

Phillips (14) observed that skimmilk, dried at elevated temperatures and then reconstituted, produced inferior cottage cheese.

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Reid and Maughan (16) found that they could manufacture cottage cheese of satisfactory quality from NDM by reconstituting to 20% total solids and using 12 ml. of rennet per 1,000 lb. of skimmilk. Wilkowske (27) estimated that the pH of the curd at the recommended cutting acidities of Reid and Maughan fell between pH 4.9 and 5.4; this is higher than the pH normally used for cutting cottage cheese. Reid and Maughan probably based their observations on spray-dried NDM containing denatured whey proteins in excess of the amounts acceptable by the standards of Morris et al. (13).

The addition of calcium chloride has been recommended by many investigators (6, 10, 15, 20, 21, 25, 26), to aid in overcoming the adverse effects of overheating skimmilk for cottage cheese.

This paper is a report of studies on the manufacture of cottage cheese from skimmilk which had been overheated either before or after concentrating and of attempts to improve the cheese by using calcium chloride, controlling the acidity at cutting with the A-C test, and varying the amounts of rennet.

EXPERIMENTAL PROCEDURES

Cottage cheese manufacture. Cottage cheese was made by two methods:

(a) Small vats with a capacity of 55 lb. were used to make 25 to 40 lb. of skimmilk into cottage cheese by the short-set method, using 5% lactic culture and a setting temperature of 90° F. Rennet was added at the indicated levels 1½ hr. after the starter. The cutting time was determined by the A-C test (5), unless indicated otherwise. The curd was cut into ½-in.-square columns, to make the large-curd or flake-type cottage cheese. Fifteen minutes after cutting, water at 120° F. was gently poured over the curd at the rate of 25% of the original skimmilk. The curd was always heated to 118° F. in 1 hr. and 40 min. from cutting, and maintained at 118° F. for an additional 20 min. before draining. Three wash waters were used at 70, 50, and 50° F., respectively; each equaled the volume of the original milk. The washed curd was drained overnight at 50° F.

(b) Sixty-gallon experimental vats were used for making 300-lb. lots of skimmilk into cottage cheese by the method used in the smaller vats until cutting. The curd was cut into ½-in. cubes; 15 min. later, heat was applied to raise the temperature to 103° F. in 45 min.; the final cooking temperature of 125 to 140° F. was reached approximately 2 hr. after cutting. The end of cooking and the time of dipping were determined by measuring curd firmness (4) at 60–70° F. After removing the whey, the curd was washed three times as in method *(a)*, drained in the vat for 2 hr., then weighed and salted at the rate of 1%.

Grading and scoring. Two samples of salted curd from each vat were placed in cottage cheese cartons. One sample was creamed to 4% fat content with cottage cheese dressing containing 12% fat. Judges graded each sample of curd—creamed and uncreamed—for body characteristics and uniformity of particle size. The following grading system was used: 1.0—Excellent, 2.0—Highly desirable, 3.0—Satisfactory, 4.0—Objectionable, 5.0—Very objectionable, 6.0—Unfit for sale. An excellent curd was very uniform in particle size, firm and meaty in body, and tender and smooth when crushed in the mouth. The

judges' scores on both creamed and uncreamed samples were averaged to give the final grades.

Ratings of commercial products by this scale of grading were obtained by submitting to the judges unidentified samples of popular brands of cottage cheese sold in Madison, Wisconsin. These samples were submitted to the judges as were all other experimental lots. The rating on body of these typical commercial samples ranged from 3.0 to 3.7 and on uniformity of particle size, from 2.8 to 3.8.

Measurements of pH. Two methods were used: *(a)* Measurements by glass electrode were made with a Model G Beckman pH meter, with external glass and reference electrodes; *(b)* a quinhydrone gold electrode and saturated calomel half-cell were used for pH measurements with a portable Leeds-Northrup potentiometer (Catalog No. 7655), according to the procedure described by Van Slyke and Price (26). Measurements of pH of centrifuged curd, from which approximately 75% of the whey had been removed, were 0.10 to 0.14 lower than in uncentrifuged curd. Under the conditions used in these experiments, the difference depended on the degree of centrifugation and the total solids of the original skimmilk.

Total solids of curd. The curd was forced through an 8-mesh sieve; 2–5 g. of the mixed curd was weighed into previously dried, cooled, and tared 50-ml. beakers. The samples were dried either in a forced-draft oven at 110° C. for 16 hr., or over boiling water for 1 hr., followed by 4 hr. in a vacuum oven at 100° C. and at least 27 in. of vacuum.

Solubility index. The solubility index of skimmilk concentrate was determined by reconstituting the concentrate to 8.8% total solids, centrifuging and treating the skimmilk in the manner recommended for reconstituted powder by the American Dry Milk Institute (2).

A.D.S.A. curd tension. The American Dairy Science Association modification (1) of the Hill curd tension test was used with 250-ml. beakers. The curd was cut with the standard knife (1), using the Cherry-Burrell curd tension meter (3).

Rennet-curd strength. Rennet-curd strength was determined with the same apparatus used for the A.D.S.A. curd tension. Curd was formed in a 250-ml. beaker by adding to 100 ml. of skimmilk, at 95° F., 1 ml. of a solution of 2 ml. of commercial rennet extract in 105 ml. of distilled water. Curd strength measurements were made 30 min. after adding the rennet.

Rennet-calcium-curd strength. Rennet-calcium-curd strength was determined in the same way as rennet-curd strength, except that calcium chloride was added to the skimmilk at the rate of 0.02% before adding the rennet.

Acid-Coagulation (A-C) test. This test, which has been described elsewhere in detail (5), is essentially the procedure of detecting the initial formation of a gel produced at the normal setting temperature by lactic culture in the absence of rennet. Gel formation in this test is identified by the first appearance of whey in cuts made by a spatula in the rennet-free, acid gel.

RESULTS AND DISCUSSION

That the A-C test performs an unusual function in selecting the optimum acidity for cutting cottage cheese curd made from overheated skimmilk was shown by these preliminary trials:

The A-C test and the time for cutting curd from "high-heat" milk. The effects of cutting cottage cheese curd at acidities higher and lower than that selected by the A-C test are shown in Table 1. All four lots of skimmilk used had been exposed at some time in processing to temperatures in excess of those normally used for pasteurization: Two of the lots were reconstituted from different samples of "bakery-type," high-heat NDM; the other two lots of skimmilk had been heated to 175° F. for 30 min. The 55-lb. vats were used, and three vats were worked simultaneously. Calcium chloride was added at the rate of 0.02% to each vat. All lots of curd were heated to a temperature of 118° F. The three vats differed only in the acidity of the curd at cutting; one vat was always cut at the A-C end-point, while the other vats were cut at either higher or lower acidities.

Table 1 shows some of the conditions and results associated with the skimmilk and curd treatments. The amount of rennet used in the skimmilk was normal in one set of three vats and above normal in the other sets because the benefits of the larger amounts had been previously observed.

The pH and titratable acidity measurements at the A-C end-points showed lower cutting acidities than usual for properly pasteurized skimmilk. The pH values of curd at cutting of the other two lots of each set of three vats ranged from 0.25 unit above to 0.22 unit below the pH at the A-C end-points; titratable acidities of the whey ranged from 0.11 below to 0.12% above the acidities at the A-C end-point. Even though all vats were cooked to the same temperature of 118° F., the total solids in the cheese from each set of three vats decreased as the acidity at cutting increased.

An analysis of differences in the scores within each set of three vats showed that the curd cut at the A-C end-point was significantly better in body and in uniformity of particle size: differences in body scores averaged 0.71, and in

TABLE 1

Effect of cutting at acidities higher and lower than the A-C end-point on quality of cottage cheese

Milk source	Total solids of skimmilk	Rennet for 1,000 lb. skim	pH[a] of curd at cutting	Titratable acidity of whey at cutting	Total solids of curd[c]	Cottage cheese quality	
						Particle size	Body
	(%)	*(ml.)*		*(%)*	*(%)*		
NDM	10.6	1.0	.....	.48[b]	17.5	2.5	2.8
("high-heat")	10.6	1.0	.....	.55	16.5	3.8	4.0
	10.6	1.0	.....	.60	14.3	4.9	4.7
NDM	11.1	3.0	5.18	.40	24.6	4.5	3.9
("high-heat")	11.1	3.0	4.93	.51[b]	21.6	2.9	2.8
	11.1	3.0	4.85	.59	20.0	2.6	2.9
Skimmilk	8.5	6.0	5.22	.29	22.0	3.1	3.1
(175° F.,	8.5	6.0	5.09	.33[b]	20.9	3.0	3.0
30 min.)	8.5	6.0	5.00	.38	19.6	3.5	3.3
Skimmilk	8.5	6.0	5.26	.30	20.9	3.8	3.6
(175° F.,	8.5	6.0	5.12	.33[b]	19.4	3.3	3.8
30 min.)	8.5	6.0	4.90	.39	17.6	4.0	5.0

[a] Measured with quinhydrone electrode on curd after centrifuging to remove approximately 75% of whey.
[b] Cut at the A-C end-point.
[c] Dried at 110° C. for 16 hr.

particle size, 0.85. These differences are significant at the 95% level of probability.

Use of calcium chloride. Calcium chloride commonly has been regarded as a useful additive in making cheese from skimmilk heated in excess of that required for pasteurization. Calcium chloride was, therefore, added at levels of 0, 0.02, and 0.05% to four different lots of skimmilk. These lots were *(a)* skimmilk heated to 175° F. for 30 min., *(b)* skimmilk heated to 145° F. for 30 min., *(c)* reconstituted commercial "low-heat" NDM, and *(d)* reconstituted commercial, "bakery-type," or "high-heat" NDM. Table 2 summarizes the conditions and results of these treatments.

The experiment was set up as a randomized complete block design, with different levels of calcium chloride as treatments and the four skimmilks as blocks. Analysis of variance showed that the addition of calcium chloride had no significant effect on the total solids of the finished curd, strength of curd at cutting, or organoleptic scores for particle size or body.

These data confirm the observations of Thurston and Gould (23), Henson and Miller (11), and Rudnick (17), that the addition of legal amounts of calcium chloride—0.02%—to skimmilk was not beneficial for making cottage cheese. This experiment does not indicate whether $CaCl_2$ would be beneficial if used in larger amounts or if the curd were cut at acidities other than those at the A-C end-point.

Effect of heat on concentrated skimmilk for cottage cheese. Rudnick (17) observed that overheating skimmilk while concentrated resulted in a fragile curd and cheese of decreased quality. This effect was studied experimentally by using four separate lots of skimmilk—A, B, C, and D. These lots were each obtained during a separate week of February and March, from the mixed milk supply of the dairy manufacturing laboratory of the University of Wisconsin. Each lot was pasteurized at 143° F. for 30 min. and concentrated in a low-temperature, single-effect vacuum pan in which the vapor temperature was kept below 86° F. The total solids of the four lots were 47.6, 43.5, 43.9, and 40.1%, respectively. Each lot of concentrate was divided into five portions; one portion from each lot was not heated; each of the other four portions of each lot were given one of the following heat treatments: 130, 140, 150, or 160° F. for 15 min. After the heat treatments, each portion was immediately cooled, diluted to 8.8% total solids with distilled water at 36° F., and stored at 36° F. for further use.

Measurements of A.D.S.A. curd tension, solubility index, Harland-Ashworth test (8), rennet-curd strength, and rennet-calcium-curd strength were determined on this skimmilk containing 8.8% total solids. Cottage cheese was made in the 55-lb. vats from each portion of skimmilk. Three vats were made each day. One of the three vats always contained the control lot of skimmilk that had received no heat treatment while concentrated. All vats were heated, as usual, to 118° F. Observations on the cheese included pH at cutting, total solids in curd, and organoleptic quality of the cottage cheese.

Table 3 lists these data for most of the heat treatments used in these experiments. To conserve space, data from those heat treatments which resulted in cheese of the same quality as that of its control lots are not shown in Table 3.

TABLE 2

Effect of the addition of calcium chloride to skimmilk on the strength of the curd at cutting, the total solids, and the quality of cottage cheese curd made from skimmilk receiving different heat treatments

Milk	Rennet for 1,000 lb. skim	Total solids in skimmilk	Anhydrous calcium chloride added	pH[a] of curd at A-C end-point	Titratable acidity of whey at A-C end-point[a]	Curd strength at cutting[b]	Total solids of curd[c]	Cottage cheese quality	
								Particle size	Body
	(ml.)	*(%)*	*(%)*		*(%)*	*(g.)*	*(%)*		
Skim heated	3.0	8.3	0.00	5.08	0.33	12	17.5	2.7	3.1
to 175° F.	3.0	8.3	0.02	5.03	0.34	12	18.0	3.0	3.0
for 30 min.	3.0	8.3	0.05	5.02	0.34	10	18.1	3.1	3.1
Skim heated	1.0	8.3	0.00	4.61	0.46	26	19.5	2.3	2.4
to 145° F.	1.0	8.3	0.02	4.61	0.46	23	19.9	2.3	2.3
for 30 min.	1.0	8.3	0.05	4.57	0.47	28	19.0	2.7	2.5
Reconstituted	1.0	10.8	0.00	4.64	0.64	100	19.5	2.3	2.4
high-heat	1.0	10.8	0.02	4.66	0.64	92	19.7	2.1	2.5
NDM	1.0	10.8	0.05	4.63	0.65	110	19.7	2.3	2.8
Reconstituted	2.0	10.9	0.00	4.95	0.47	36	19.0	3.4	3.5
high-heat	2.0	10.9	0.02	4.93	0.48	38	19.1	3.4	3.4
NDM	2.0	10.9	0.05	4.90	0.50	36	19.2	3.3	3.5

[a] Measured with quinhydrone electrode on curd after removing approximately 75% of the whey by centrifuging.
[b] Strength of the acid-rennet cottage cheese curd in the vat at the A-C end-point.
[c] Dried in forced-draft oven at 110° C. for 16 hr.

The heat treatments of the concentrates had no significant effect on the Harland-Ashworth test for undenatured whey proteins, nor upon the A.D.S.A. test for curd tension. In only Lot A, the concentrate with highest solids-not-fat, did the solubility index show any effect of heat treatment of the concentrate. Since these heat treatments are sometimes used in making nonfat dry milk, these three tests can not, in the light of these experiments, be considered acceptable for the complete evaluation of NDM to be used for the manufacture of cottage cheese. This conclusion has been verified in trials with NDM obtained from commercial sources and from the manufacturing laboratory of the University of Wisconsin.

The heat treatments of the concentrate had no significant effect upon the pH of the coagulum at the A-C end-point. These treatments had no effect on the solubility index measurements on Lots B, C, and D. The total solids content of the cheese was not affected by the heat treatments of the concentrate used.

The quality of curd in the control lots was superior to that produced from skimmilks made from Concentrates A and B after heating to 150 and 160° F., from Concentrate B after heating to 130 and 140° F., and from Concentrates C and D after heating to 160° F. In all instances, the defective curd was criticized for greater tendency toward mealiness and softness, even though percentages of total solids in these lots were practically identical to total solids in their controls.

Measurements of curd strength using rennet or rennet and calcium were the only tests that reflected the severity of the heat treatment of the concentrate before cottage cheese manufacture. Not enough trials could be made in this study to establish standards of curd strength for evaluating the heat treatments of the product to be used for manufacturing. Such standards would have to take into consideration the variable response of different milks to rennet action, and the effects of heat treatments that denature the whey proteins or affect rennet coagulation of milk.

The results obtained in this study do suggest that the rennet-calcium-curd strength test might be useful, after more study, as a line test in the manufacture of NDM for cottage cheese. This possibility is especially interesting, because the test seems to detect the effect of heat treatments on concentrated milks, even when such treatments do not cause denaturation of the whey proteins as measured by the Harland-Ashworth test.

Although heating skimmilk to 160° F. for 15 min. would denature approximately 15–20% of the serum proteins (9), this treatment had no measurable denaturing effect on the whey proteins of the concentrates heated in this study. This confirms the observations of other workers (9, 12). Zehren (28) observed that increased ionic strength and lowered pH, both of which are characteristics of skim concentrate, decreased the denaturation of beta-lactoglobulin upon heat treatment.

Since these heat treatments did not affect the whey proteins, the most likely cause of the lowered quality of the cottage cheese would seem to be in changes in the casein itself during heating.

TABLE 3

Effects of heat on four lots of concentrated skimmilk when reconstituted to 8.8% total solids and made into cottage cheese

Skim-milk lot	Total solids of concentrate	Heat treatment of concentrate for 15 min.	A.D.S.A. curd tension	Harland-Ashworth test[a]	Solubility index	Rennet curd tension	Rennet calcium curd tension	pH of curd[b] at cutting	Total solids of cottage cheese[c]	Cheese quality	
										Particle size	Body
	(%)	*(° F.)*	*(g.)*		*(ml.)*	*(g.)*	*(g.)*		*(%)*		
A	47.6	Control	53	6.8	0.1	..	....	4.75	24.6	2.1	2.3
	47.6	150	51	7.0	0.8	..	....	4.75	25.0	3.3	3.4
	47.6	160	49	6.9	1.3	..	....	4.75	24.8	3.5	4.0
B	43.5	Control	44	6.6	0.1	0	21	4.71	21.1	2.1	2.3
	43.5	130	47	....	0.1	0	17	4.71	21.1	2.4	2.3
	43.5	140	43	....	0.1	0	16	4.72	20.5	2.8	2.6
B	43.5	Control	44	6.6	0.1	0	21	4.72	21.4	2.3	2.3
	43.5	150	48	6.6	0.1	0	10	4.70	21.3	3.3	2.9
	43.5	160	48	6.6	0.1	0	5	4.72	21.5	4.5	3.4
C	43.9	Control	50	6.6	0.1	10	42	4.71	20.7	2.1	2.8
	43.9	150	64	6.5	0.1	1	25	4.72	20.7	2.1	2.7
	43.9	160	58	6.6	0.1	0	19	4.72	20.6	2.4	3.0
D	40.1	Control	51	6.5	0.1	18	57	4.76	25.3	1.9	2.1
	40.1	150	55	6.6	0.1	7	42	4.75	24.4	1.8	2.0
	40.1	160	46	6.5	0.1	2	30	4.75	25.1	2.1	2.3

[a] Milligrams nitrogen/grams total solids.
[b] Measured with glass electrode on uncentrifuged curd at the A-C end-point.
[c] Dried in forced draft oven at 110° C. for 16 hr.

These data and the observations of Rudnick (17) indicate that much of the difficulty in the use of nonfat dry milk for cottage cheese may be caused by overheating the skimmilk while in the concentrated form. The results of cottage cheese manufacture from some lots of commercial nonfat dry milk and from nonfat dry milk manufactured in our laboratory further substantiate this last observation.

Effects of heating skimmilk to 175° F. for 30 min. Preliminary work with the A-C test had indicated that increased amounts of rennet might be used to improve the quality of cottage cheese made from skimmilk which had been heated to temperatures that denature whey proteins. A heat treatment of 175° F. for 30 min. (high-heat) for skimmilk was arbitrarily selected for comparison with a control lot of skimmilk heated to 143° F. for 30 min. (low-heat).

The experimental treatments began by dividing one lot of skimmilk into two portions for the two heat treatments in each trial. Varied rennet additions were tested at levels of 1, 5, 10, 15, and 20 ml. per 1,000 lb. of the high-heat skimmilk; 1 ml. of rennet per 1,000 lb. was used in the control lot of low-heat skimmilk. Large-curd cottage cheese was manufactured in five trials of four 500-lb. vats each. In each trial, the control vat contained low-heat skimmilk and the other three vats contained identical milk which had received the high-heat treatment. Each of the three high-heat vats was made with a different amount of rennet. The five levels of rennet were applied so that each was used three times in the five trials. Table 4 lists data for curd strength at cutting and scores

TABLE 4

Effect of different levels of rennet on the manufacture of cottage cheese from skimmilk heated to 175° F. for 30 minutes

Trial No.	Rennet per 1,000 lb. of skimmilk heated to 175° F. for 30 min.					Rennet per 1,000 lb. of skimmilk heated to 145° F. for 30 min.
	1 ml.	5 ml.	10 ml.	15 ml.	20 ml.	1 ml.
	Curd strength at cutting at A-C end-point[a]					
1	12	....	26	26	....	?
2	..	26	25	....	28	90
3	8	20	....	25	....	106
4	..	30	30	....	30	90
5	8	....	....	26	28	88
	Body score—organoleptic					
1	3.25	.....	3.0	2.95	.....	2.7
2		3.35	3.3	.....	3.15	2.65
3	3.8	2.8	....	2.9	.....	2.1
4	.....	3.4	3.1	.....	2.8	2.5
5	2.85	.....	.....	2.8	2.65	2.95
	Uniformity of particle size—organoleptic					
1	3.3	.....	3.15	2.4	.....	3.0
2		3.8	3.35		3.0	2.75
3	4.35	2.85	.....	2.85	....	2.0
4	.....	3.65	3.4	....	2.85	2.5
5	3.25	.....	.....	3.0	2.65	2.65

[a] Curd strength of the several lots at cutting at the A-C end-point was measured with A.D.S.A. curd tension apparatus on coagulum formed in the 250-ml. beaker by the action of the starter and the varied amounts of rennet at vat setting temperature.

of body and particle uniformity associated with each variation of the experimental conditions.

Curd strength observed in curd made from high-heat milk with 5 to 20 ml. of rennet per 1,000 lb. was, in general, less than one-third of the curd strength shown by the coagulum of the low-heat milk; with 1 ml. of rennet in high-heat milk, the curd strength was approximately one-tenth that of the control. The three lower levels of rennet were definitely unsatisfactory in the high-heat milk. The use of 15 and 20 ml. per 1,000 lb., however, gave curd which, although slightly inferior to that made with low-heat milk, still compared favorably with the quality of commercially available products. In general, the judges observed mealiness and lack of uniformity in particle size more often in curd from the high-heat than from the low-heat skimmilk.

Table 5 summarizes data obtained in the manufacture of curd from the high-heat and low-heat skimmilks. Yield data do not include curd made from high-heat milk with 1 ml/1,000 lb. rennet. The evidence indicates a disadvantage and certain advantages in using high-heat skimmilk for the manufacture of cottage cheese:

The pH of 5.05 of the curd from the high-heat skimmilk is considered to be too high for satisfactory keeping quality of the creamed curd; the pH 4.87 of the curd from the low-heat skimmilk is better. A specialized procedure for manufacture or acidification might eliminate these defects.

Use of the high-heat skimmilk shortened by 1 hr. the time from adding the starter to cutting. The average yield of curd per 100 lb. of low-heat skimmilk was

TABLE 5

Summary of important observations on the manufacture of cottage cheese from skimmilk heated to 175 and to 143° F. for 30 min.

Observations	Skimmilk heated to 175° F. for 30 min.		Skimmilk heated to 145° F. for 30 min.	
	Average[a]	Range	Average[b]	Range
1. Time from adding starter to cutting *(hr.)*	3.85	3.17– 4.33	4.83	4.08– 6.00
2. pH of curd at cutting	5.13	5.02– 5.23	4.72	4.68– 4.76
3. Titratable acidity of whey at cutting *(%)*	0.33	0.32– 0.37	0.45	0.42– 0.48
4. Cooking temperatures *(° F.)*	133.6	124–143	135.8	132–140
5. pH of uncreamed salted curd	5.05	4.93– 5.12	4.87	4.87– 4.92
6. Curd firmness *(g.)*	175	155–220	184	161–217
7. Total solids of curd *(%)*[c]	20.45	19.4 –23.0	19.98	19.3 –21.5
8. Yield of curd per 100 lb. skimmilk and starter *(lb.)*	15.35	13.6 –16.8	13.90	12.4 –14.9
9. Adjusted yield of curd *(lb.)*[d]	15.85	15.1 –17.2	14.10	13.2 –14.7
10. Per cent recovery of skimmilk solids in cottage cheese *(%)*	36.02	34.2 –39.1	32.0	30.0 –33.3

[a] Averages of 15 vats for all observations except 8, 9, and 10, which omit three lots made with 1 ml/1,000 lb. rennet.

[b] Average of five control vats.

[c] Dried over boiling water for 1 hr., then at 100° C. under 27.5 in. vacuum for 4 hr.

[d] Yield calculated on basis of 20% total solids in curd made from 100 lb. of skim and starter containing 8.8% SNF.

13.90 lb., from the high-heat skimmilk, 15.35 lb., an average increase of 10.4%. When the yields of curd were adjusted to 20% total solids from skimmilk with 8.8% total solids, the increase in yield per 100 lb. of skim was from 14.10 to 15.85 lb., an increase of 12.4%. The percentage of skimmilk solids recovered in cottage cheese made from low-heat skim was 32.0%, but the recovery of solids from the high-heat skimmilk was 36.0%.

The increased yields were almost certainly due to the retention of whey proteins in the curd. This increase in yield from milk heated to temperatures that denature the whey proteins parallels the observations of Schwarz and Mumm (19), Tretsven (24), and Sadek and Motteleb (18). The prospect of increasing yields by heat treatments to include denatured whey proteins in the curd has an attractive economic significance for the cottage cheese industry.

Little difference was noted in the effect of the two heat treatments on cooking temperatures, total solids, or curd firmness; nor were there any differences in the time required for cooking that could be attributed to the heat treatment of the skimmilk.

It may be startling to the experienced cheese-maker to think that acceptable cheese was made from skimmilk heated to 175° F. for 30 min. These results were made possible only by using increased amounts of rennet in conjunction with the A-C test, which detects the optimum condition of the curd for cutting. No difficulty was found in expelling whey from the curd during heating or in obtaining desired firmness and total solids in the finished curd. Difficulty would certainly have been encountered had the high-heat vats been cut at the same acidities as the low-heat, control vats.

This cottage cheese–making procedure has not been tested on skimmilk exposed to ultra-high temperature, short-time pasteurization.

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STAPHYLOCOCCUS AUREUS IN CHEDDAR CHEESE [1]

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SUMMARY

Experiments were carried out to determine the factors affecting the growth of staphylococci in raw milk and Cheddar cheese. Milk with a relatively low standard plate count was found to support an active growth at 32° C. and a moderate growth at 22° C.; whereas, almost no growth took place, even at 32° C., in milk with a high standard plate count. Large numbers of staphylococci in milk would be most apt to occur when bacteriologically clean milk is held at a fairly high temperature. Staphylococci grew poorly during cheese-making and most of them were concentrated in the curd. The initial staphylococcus count of milk seems to be the most important factor determining the number of staphylococci in cheese. Prompt cooling of milk after milking and heat treatment before cheese-making are suggested to minimize any public health hazard associated with the presence of staphylococci in cheese.

It has been reported by Thatcher *et al.* (8) that cheese made in Canada sometimes contained large numbers of toxigenic staphylococci. The presence of large numbers of staphylococci in cheese must be due either to gross contamination or to incidental contamination, followed by growth either in the milk or cheese or in both.

This paper reports on the factors affecting the multiplication of staphylococci in raw milk and during various phases of cheese-making. The results obtained in a survey of staphylococci in Cheddar cheese made from raw milk and heat-treated milk also will be presented.

METHODS

Enterotoxigenic strains[3] of *Staphylococcus aureus* L16, S6, and 161C were used for making artificially infected milk. These cultures were maintained on nutrient agar supplemented with 1% yeast extract. Cultures grown in nutrient broth with 1% yeast extract for 18 hr. at 37° C. were diluted in sterile skimmilk. One milliliter of diluted culture was added to 100 ml. of raw milk. Both Central Experimental Farm milk and commercial bulk milk were used. The Standard Plate Count (SPC) (1) of milk at the time of inoculation ranged from 3,300 to 5,500,000 per milliliter. The milk samples with a SPC of over 5,000,000 per milliliter were obtained by incubating low-count milk at room temperature for 6 to 8 hr. Duplicate portions of samples were incubated at each of 22 and 32° C. for 16 hr. Samples of milk were also incubated without artificial infection, to

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[3] Strain L16 was supplied by Dr. F. S. Thatcher of the Food and Drug Directorate, Department of National Health and Welfare, Ottawa, and S6 and 161C by Dr. E. Wilson of the U. S. Public Health Service Laboratory in Cincinnati.

determine the growth rate of staphylococci naturally present in milk. Mannitol-salt agar (Difco) was used for making staphylococcus counts. All plates were incubated at 37° C. for 48 hr. Representative mannitol-positive colonies were then subjected to the coagulase test. Estimates of staphylococci were based on the proportion of colonies that were coagulase-positive.

Cheese was made from Central Experimental Farm raw milk (SPC 2,200 per milliliter) and commercial bulk milk (SPC 1,600,000 per milliliter) by a conventional Cheddar cheese-making process. Staphylococci (Culture L16) were added to raw milk at the time of adding the starter culture, except in Trial V, in which the growth rate of naturally present staphylococci was determined. Since it was found that 2% sodium citrate did not alter the viability of *S. aureus* over a short period of time, cheese and curd samples were homogenized in the citrate solution with the Waring Blender for 3 min.; estimates of staphylococci were then made by the method described for milk samples.

RESULTS

Growth of staphylococci in raw milk. Since no appreciable difference in the rate of multiplication was noted between Cultures L16, S6, and 161C, Table 1 shows only the results obtained with Culture L16. The rate of multiplication of staphylococci naturally present in raw milk also is summarized in Table 1. As expected, *S. aureus* grew better at 32° C. than at 22° C., both in artificially and in naturally infected milks. However, the rate of multiplication at both temperatures appears to be inversely correlated with the initial SPC of milk. Almost no growth took place, even at 32° C., when the SPC of milk was very high. Thus, the number of bacteria other than staphylococci seems to be one of the limiting factors influencing the growth of staphylococci in raw milk. This is supported further by the following experiment. When milk with SPC of 260,000 per milliliter, in which *S. aureus* multiplied 120-fold at 32° C. in 16 hr., was inoculated with Culture L16, together with 2% commercial starter culture, no increase in staphylococcus count was observed after 16 hr.

TABLE 1

Growth of *Staphylococcus aureus* in raw milk

Milk sample No.	SPC (10^3/ml) of milk 0 hr.	Staphylococcus counts (10^3/ml)					
		Artificially infected milk (Strain L16)			Naturally infected milk		
		0 hr.	32° C. 16 hr.	22° C. 16 hr.	0 hr.	32° C. 16 hr.	22° C. 16 hr.
1	0 (autoclaved)	5	58,000 (×12,000)[a]	740 (×150)	.....	.....	.....
2	3.3	7	25,000 (× 3,600)	130 (× 19)	0.53	85 (×160)	5.9 (×11)
3	260	12	1,400 (× 120)	42 (× 3.5)	0.55	56 (×100)	2.7 (×4.9)
4	5,100	10	<100 (×<10)	15 (× 1.5)	2.1	7.5 (×3.6)	5 (×2.4)
5	5,500	7.9	10 (× 1.3)	9.5 (× 1.2)	0.75	1.4 (×1.9)	1 (×1.3)

[a] Numbers in parentheses represent the extent of multiplication.

Growth of staphylococci during cheese-making. Changes in staphylococcus counts in the milk, whey, and curd during cheese-making are shown in Table 2. In no case was a significant change in staphylococcus count observed during the period before the rennet was added. However, at the end of cooking a decided increase in number in the curd was noted with both added and naturally present staphylococci. Only a slight growth took place thereafter until hooping, although the temperature in the curd (102 to 90° F.) was favorable for the growth of staphylococci. Highest counts were found in curd at the time of hooping. If one assumes that the number of staphylococci in whey is constant, and that the yield of cheese is 10% of the weight of milk used, the rate of multiplication may be calculated by the following formula:

$$\text{Rate of multiplication} = \frac{\left(\begin{matrix}\text{Count in curd}\\ \text{at hooping}\end{matrix}\right) \times 10 + (\text{Count in whey}) \times 90}{(\text{Count in milk at 0 hr.}) \times 100}$$

In Trials I and II, where milk with very low SPC was used, staphylococci multiplied about eightfold, and about fourfold in Trials III and IV, where commercial bulk milk of relatively high SPC was used. Naturally present staphylococci (Trial V) multiplied only twofold during cheese-making.

TABLE 2

Growth of *Staphylococcus aureus* during cheese-making

Time of sampling	Trial No. I	II	III	IV	V
	Staphylococcus counts (10^3/ml or gram)				
Milk at 0 hr.	22	24	24	12	0.8
Milk at renneting	19	24	25	16	0.9
Whey at the end of cooking	2.1	1.1	2.4	2.1	0.14
Curd at the end of cooking	840	700	660	430	12
Curd at hooping	1,900	1,700	1,100	460	16
Cheese (1 day)	1,600	1,400	810	340	12
Cheese (2 mo.)	680	1,200	99	45	0.9
SPC (10^3/ml) of milk at 0 hr.	2.2	2.2	1,600	1,600	1,600

Whey after cooking contained very few staphylococci, suggesting that most of them were trapped in the curd. This would imply that, even if no growth takes place during cheese-making, the count of the resulting cheese would be roughly ten times higher than the count of the original milk.

The staphylococcus count in cheese decreased gradually during curing and storage. The curing and storage period consisted of 2 wk. at 58° F., followed by 6 wk. at 40° F. After 2 mo., the counts in cheese made from high SPC milk dropped by about 90%, whereas cheese made from milk of very low SPC still contained fairly large numbers of staphylococci.

Staphylococcus count in Canadian Cheddar cheese. The survey of staphylococci in Cheddar cheese samples commenced in April and was concluded in November, 1958. Most samples were collected randomly at various grading stations in the Provinces of Ontario and Quebec, with a few samples from other

provinces. Samples were 2 to 3 wk. old when they were plated. Of 333 phosphatase-positive samples, 119 (36%) contained staphylococci in excess of 10,000 per gram. A marked seasonal effect is evident, higher counts reflecting warmer weather conditions (Table 3). If counts in excess of 500,000 per gram are considered dangerous (2), about 6% of the samples fall into this category. These were all made during the period June to September. On the other hand, counts for 19 out of 20 phosphatase-negative samples, presumably made from pasteurized milk, were less than 100 per gram. The other phosphatase-negative sample had a count of 18,000 per gram.

TABLE 3

Seasonal variation of staphylococcus count in Canadian Cheddar cheese made from raw milk[a]

Month	No. of samples examined	% of samples containing more than 10,000/g	% of samples containing more than 500,000/g	Highest count (10^3/g)
April	26	0	0	2.7
May	60	18	0	300
June-July	60	72	25	6,700
August	41	59	10	4,100
September	47	36	4	1,100
October	37	24	0	140
November	62	24	0	84
Total	333	36	6	

[a] Phosphatase-positive samples.

Heat treatment of milk. A study was made to determine the effectiveness of milder heat treatment of milk in reducing staphylococci in cheese. Samples were collected at a nearby factory from ten lots of milk flash-heated at 150° F. Before heat treatment, staphylococcus counts of milk ranged from 0 to 2,300 per milliliter, with an average of 510 per milliliter. No staphylococcus colonies were developed on mannitol-salt agar when 0.1 ml. of heated milk was plated. Seven curd samples made from the heated milk were found to be free from staphylococci when 0.1 g. was plated. Three samples contained 300 staphylococci per gram, suggesting a slight recontamination in the cheese vats.

Prompted by the high staphylococcus counts in raw milk cheese made in June to September, samples of cheese made from heat-treated milk during the same period were subsequently collected and examined for staphylococci. These cheese samples were 2 to 3 mo. old when examined. Of 35 samples, 33 gave negative results when 0.1 g. was plated. The counts for two positive samples were 550 and 1,700 per g. The percentage of negative samples in cheese made from heat-treated milk was comparable to that in cheese made from pasteurized milk.

DISCUSSION

A negative correlation has been observed between the initial SPC of milk and the extent of multiplication of staphylococci. This is in agreement with the findings of Gibson and Abd-El-Malek (3), that staphylococci at low temperatures (10 to 20° C.) can grow actively only when the total bacterial count is low.

Smith (7) and Jones *et al.* (6) have also stated that large numbers of staphylococci in raw milk are unlikely to occur unless milk from infected udders is withdrawn under hygienic conditions and kept at a fairly high temperature. Heinemann (4) has reported that three enterotoxigenic strains of *S. aureus* grew poorly at temperatures of 80° F. or lower. His findings, however, might be attributed to the fact that the milk used in his experiments had relatively high bacterial counts, averaging 1,270,000 per milliliter. It is apparent that the number of bacteria other than staphylococci, and the holding temperature of milk, are important factors determining the growth of staphylococci in milk.

In our experimental cheese, staphylococci multiplied about eightfold in the vats with a low SPC milk and about fourfold with a relatively high SPC milk; most of them were found in the curd. Since the growth of staphylococci is limited during cheese-making, the number of staphylococci in cheese is determined largely by the initial count of milk.

It seems possible that the high staphylococcus counts in raw milk cheese made during the summer months may be due to the growth of staphylococci in the milk before arriving at the cheese factory. One recent survey (5) showed about 50% of milk samples to have a SPC of under 200,000 per milliliter during the warmer months. Also, much of the night's milk, in this survey, was poorly cooled; over 10% arrived in excess of 70° F. It is obvious that staphylococci would have even higher temperatures for growth, and less competition from other organisms, during the interval between the evening milking and arrival at the factory. It would appear that temperature and bacteriological conditions of milk on the farm are frequently favourable to the growth of staphylococci and could account for the high counts in the raw milk cheese.

According to the results of Heinemann (4), the thermal death time of staphylococci in milk is 1.9 min. at 150° F. In the cheese factory, however, flash heating at 150° F. was found to destroy all staphylococci naturally present in the milk. Furthermore, none of the cheese samples made from heat-treated milk contained any significant number of staphylococci. These results suggest that the public health hazard associated with the presence of staphylococci in cheese can be considerably reduced by the use of heat-treated milk for cheese-making.

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STUDIES ON MILK POWDERS. III. THE PREPARATION AND PROPERTIES OF MILK POWDERS CONTAINING LOW-MELTING BUTTER OIL[1]

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SUMMARY

Butter oil was fractionated to obtain products melting at 19–21, 22–24, and 28–30° C. Agglomerated milk powders were prepared, each containing one of these fat fractions, and one powder was prepared containing the unfractionated butter oil.

The wettabilities and dispersibilities of the four products were compared with those of commercial whole milk powder and commercial instant skimmilk powder. The results showed that the melting point of the fat in a milk powder affects greatly its wettability and dispersibility. In particular, the milk powder which contained the butterfat fraction melting at 19–21° C. had approximately the same wettability and dispersibility as commercial instant skimmilk powder.

The results suggest that control of the physical properties of the fat component of a dried milk powder provides a means of preparing a milk powder with the fat content of whole milk powder and the wettability and dispersibility of a superior grade of instant skimmilk powder.

The problem of preparing milk powders that will disperse readily in cold water has attracted widespread attention in recent years. Skimmilk powders can be made readily dispersible in water by a process commonly known as instantization. A recent patent (5) describes an instantization process for skimmilk powder whereby the milk powder is continuously moistened causing the particles to adhere together in the form of light, porous, random aggregates of a size substantially larger than the size of the original milk particles. The excess moisture is then removed from these aggregates to provide a final product that has a moisture content of less than 6%. In this connection, it may be recalled that the legal standard in the U.S.A. requires a moisture content of less than 5% in the final product. However, it has been found that the application to whole milk powder of the method (5) does not yield a readily dispersible product.

Ashworth and Bendixen (1) have claimed that the physical state of the casein is a factor limiting the rate of dispersion of milk powder. They demonstrated that precondensation of the fluid milk to 40% total solids before spray-drying produced a powder which dispersed much more readily than did one made from the same milk precondensed to 20% total solids.

Stone *et al.* (6) measured the self-dispersion of milk powders which varied in fat content from 7.5 to 48.3%. They found that self-dispersion decreased as the fat content of the powders was increased. Experiments in which the temperature of the water varied showed that milk powder dispersed more readily in

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water at a temperature just above the melting range of the fat. They attributed the rapid self-dispersion of a modified milk powder, in which the butterfat had been replaced by corn oil, to the presence of liquid oil at the temperature of the water in which the powder was dispersed.

Bullock and Winder (3) observed that whole milk powder, when first removed from the drier, exhibits a greater wettability than the same powder after storage at room temperature for a short period. Subjection of the stored powder to a post-drying heat treatment improved the wettability, and in some instances this improvement was not lost after months of storage at room temperature.

Experiments in our laboratory have confirmed those observations of Bullock and Winder (3) which relate to the wettability of freshly prepared whole milk powder. It was observed in the course of this work that milk powder prepared from summer milk is much more wettable than powder made from winter milk. This is suspected of being a consequence of the lower melting point of the fats in summer milk.

A research program has been initiated in this laboratory with the aim of developing a stable dried whole milk powder, which will reconstitute readily in cold water to give a product with the flavour and appearance of fresh fluid milk. The present paper deals with the preparation and properties of milk powder containing low-melting butter oil.

EXPERIMENTAL PROCEDURE

Preparation of low-melting butter oil.

(a) By acetone segregation.

Butter oil (8,000 g.) (summer milk), which was obtained from a local dairy, was dissolved in acetone (30 liters) at 37° C. The solution was maintained at 5° C. for a period of 12 hr. The insoluble fat was removed by the centrifugation and the acetone-soluble fraction was recovered by evaporation of the acetone under reduced pressure. The product (F_1) weighed 6.32 kg. and had a melting point of 19–21° C.

(b) By fractional crystallization.

Butter oil (8,000 g.) (winter milk), which had been obtained from a local dairy, was melted and was then maintained at a temperature below the melting point of the fat for the desired period of time. The solid fat which separated was removed from the liquid fraction by centrifugation. When a lower-melting fraction was desired, the liquid fat was again cooled at a lower temperature than was employed in the first crystallization. Table 1 gives the conditions employed in the preparation of two butter oil fractions (F_2, F_3) and the yields and melting points of these fractions.

Preparation of dried milk.

Fresh skimmilk was heated to 145° F. A sufficient quantity of butter oil was added to the milk to give a final dried product containing approximately 25% fat and the mixture was homogenized at 3,000 lb. pressure. The resulting material was evaporated in a vacuum pan to 42% solids and was then dried in a

Scott Spray Drier operating under the following conditions: inlet temperature, 318–330° F., outlet temperature, 160–170° F.

Four lots of whole milk powders were prepared by the procedure outlined above. Product 1 contained Butter Oil F_1, which was prepared by acetone segregation. Product 2 contained Butter Oil F_2, Product 3 contained Butter Oil F_3, and Product 4 contained unfractionated butter oil (winter milk) (F_4). The four products were instantized in a Blaw-Knox Instantizer (Series A 150, Series 103, Year 1957, Blaw-Knox Company, Dairy Equipment Division, Mora, Minnesota) operated under the following conditions: inlet temperature—260° F., outlet temperature—160–170° F., steam pressure on jets, 20 lb., Syntron feeder set at 90. Table 2 shows the results of the analyses of fat and total solids (Mojonnier) of the four products.

Wettability and dispersibility of the milk powder.

The wettabilities and dispersibilities of the milk powders in water were measured by methods described elsewhere (2). The powders were stored for a period of 48 hr. before the measurements on the powders were made. Each wettability result reported in Table 3 represents the average of four measurements and each dispersibility result the average of three measurements. The wettability and dispersibility of samples of commercial milk powders (whole milk powder, skimmilk powder, instant skimmilk powder, agglomerated whole milk powder manufactured in France) are also shown for the purpose of comparison.

Previous experiments (2) showed that the wettability and dispersibility of an agglomerated milk powder varies with the particle size of the powder.

It was desirable in the present study to measure the differences in wettability and dispersibility of the powders prepared with the various samples of butter oil, without interference due to particle size. The powders were sifted, therefore, and the fractions which passed through a 1.0-mm. sieve and did not pass through a 0.5-mm. sieve were chosen for the measurement of wettability and dispersibility. The temperatures of the water and the powder in these experiments were 24° C. Figure 1 shows typical curves which were obtained in the measurement of wettability of this fraction of the various laboratory-prepared powders and commercial agglomerated whole milk powder, as well as ungraded commercial whole milk powders. The difference between the area under the curve for a given powder and the area of the rectangle formed by the axis of ordinates (0–100) and the axis of abscissae (5–60) was expressed as a percentage of the rectangle, and this figure was adopted as a measure of the wettability of the powder in question.

Samples of the six size-graded powders plus the ungraded commercial whole milk powder were held at various temperatures (16–36° C.) for 48 hr. and their wettability and dispersibility in water at the same temperature as the powders were then measured. Figures 2 and 3 show in graphic form the effects of temperature on wettability and dispersibility, respectively.

Preliminary experiments suggested that the wettability and dispersibility of a powder is to a great extent dependent on the melting point of the fat component. To check this point, butter oil which was obtained from a local dairy

TABLE 1

Yields and melting points of butter oil fractions

Fat fractionated	Weight of fat	Fractionation temperature	Fractionation time	Yield of liquid fraction
	(g.)	*(° C.)*	*(hr.)*	*(g.)*
Butter oil (F_4) (winter milk)	8,000	28	18	6,500 (A)
A	6,560	22	5	4,789 (F_2) MP 22–24° C.
Butter oil (winter milk)	8,000	28	20	6,400 (F_3) MP 28–30° C.

TABLE 2

Composition of the milk powders

Product	Fat	Total solids
	(%)	
1	24.5	95.1
2	24.8	96.4
3	24.2	96.1
4	25.3	95.8

TABLE 3

Wettability and dispersibility of various milk powders

Product	Wettability		Dispersibility	
	Ungraded powder 25° C.	Particle size 0.5–1.0 mm., 24° C.	Ungraded powder 25° C.	Particle size 0.5–1.0 mm., 24° C.
	(%)			
Product No. 1 (MP of fat 19–21° C.)	87	88	80	80
Product No. 2 (MP of fat 22–24° C.)	60	62	63	45
Product No. 3 (MP of fat 28–30° C.)	40	43	46	37
Product No. 4 (MP of fat 33–35° C.)	26	20	28	20
Commercial skimmilk powder (non-inst.)	51		54	
Commercial instant skimmilk powder (I.S.M.P.)	89	90	82	81
Commercial whole milk powder (W.M.P.)	13		16	
Commercial agglomerated whole milk powder (A.W.M.P.)	2.9	4.7	4.5	4.9

was fractionated to give products having the following melting points: 19–21 (F_1), 22–24 (F_2), and 28–30° C. (F_3). Milk powders containing these fats were prepared and one powder was prepared containing the unfractionated butter oil (F_4). The powders contained approximately the same proportion of fat. The dispersibility and wettability measurements were made on the foregoing four milk powders as well as on four samples of commercial milk powder. The dispersibility and wettability values of the eight samples of milk powder are

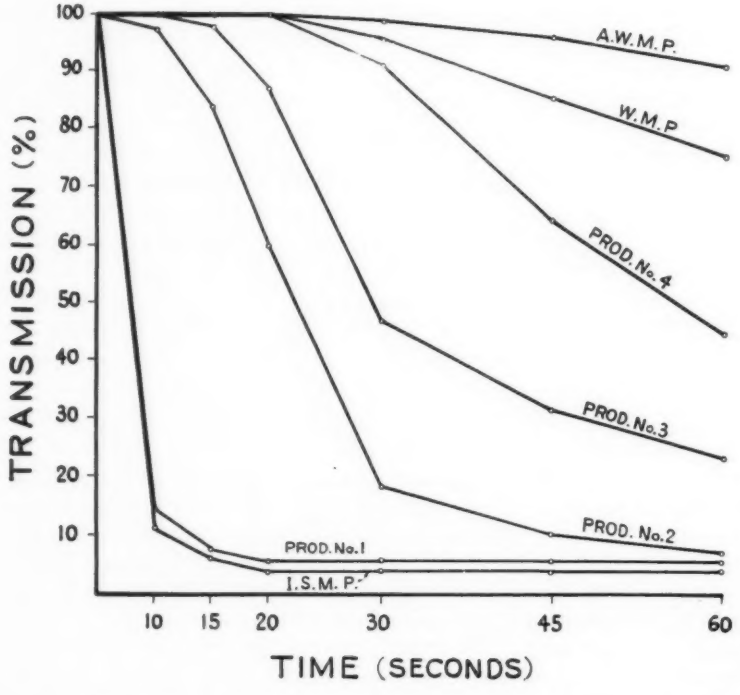

FIG. 1. Typical curves obtained in the measurement of the wettability of various milk powders.

presented in Table 3. It may be seen that the dispersibility and wettability of Product 1, which contained fat melting below 21° C., were approximately the same as those obtained with commercial instant skimmilk powder. As the melting point of the fat component increased, the dispersibility and wettability of the corresponding milk powders decreased. The results reported in Figures 2 and 3 show that the temperature of the powder and of the water affected greatly the dispersibility of the powders containing fat, but had little effect on the skimmilk powder.

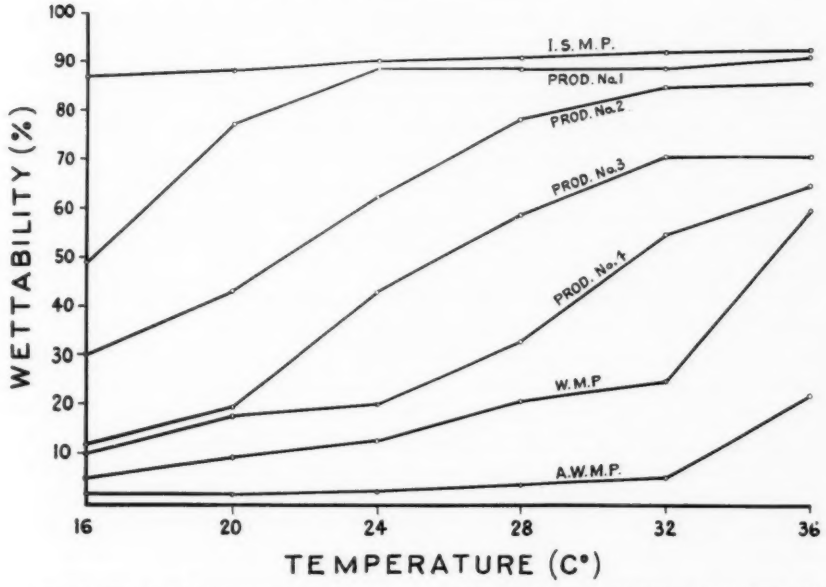

FIG. 2. Effects of temperature of the water and the powder on wettability of various milk powders.

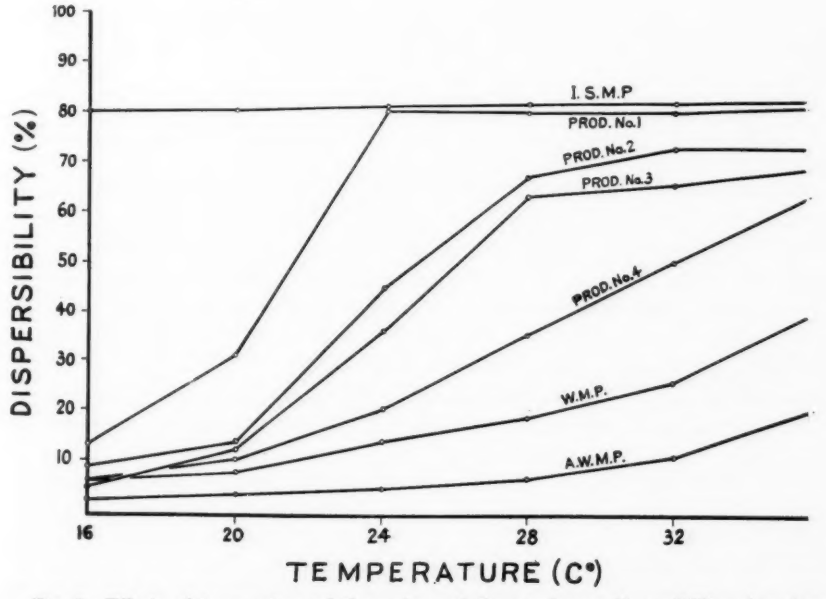

FIG. 3. Effects of temperature of the water and the powder on dispersibility of various milk powders.

DISCUSSION

It is of some interest to compare the relationship of wettability and of dispersibility to the temperatures at which the fat component of the powder in question is just liquefied. Product 1 had a wettability of approximately 80% at 21° C., the temperature at which its fat component was just liquefied; whereas, the wettabilities of Products 2, 3, and 4 were approximately 60, 65, and 62% for fat liquefaction temperatures of 24, 30, and 35° C., respectively. Clearly, the wettability did not bear a simple relationship to the circumstance that the fat component was liquid.

On considering the dispersibilities of these same products at those temperatures at which their fat components were just liquefied, it was found that Products 1 and 2 had dispersibilities of 43 and 45%, respectively, whereas Products 3 and 4 had dispersibilities of 65 and 60%, respectively. Moreover, neither the dispersibilities nor the wettabilities of Products 2, 3, and 4 reached values as high as did those of Product 1, even when the temperature was raised considerably above the melting point of the fat component of the product in question. It may be recalled here that the low-melting fat component of Product 1 was prepared by acetone fractionation. This may provide a sharper separation of low-melting fats from higher-melting fats than simple temperature fractionation, and this offers a possible explanation of the superior properties of Product 1.

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A METHOD FOR STANDARDIZING THE BIACETYL CONTENT OF CREAMED COTTAGE CHEESE [1]

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SUMMARY

The addition of 0.15% citric acid to creamed cottage cheese held at 45° F. did not increase the biacetyl content; whereas, addition of *Streptococcus citrovorus* gave a slight increase and addition of both citric acid and *S. citrovorus* gave still more of an increase. No biacetyl was formed in a 12% fat creaming mixture containing *S. citrovorus* or *S. citrovorus* plus 0.15% citric acid. Considerable biacetyl was produced in a creaming mixture containing large numbers of *S. citrovorus* after it was adjusted to pH 4.2 with citric acid and held at 70° F. Cottage cheese creamed with such a preparation had a high initial biacetyl content.

The most satisfactory method of preparing a creaming mixture which contains sufficient biacetyl and volatile acid to influence the flavor of creamed cottage cheese is the following: Heated skimmilk is inoculated with a pure culture of *S. citrovorus* and, after sufficient growth, adjusted to pH 4.3 with sterile citric acid. Further incubation at 70° F. results in the formation of considerable biacetyl and volatile acid. The skimmilk culture thus prepared is used to standardize the fat content of cream to about 12%. Standardization of biacetyl is accomplished by varying the fat content of the cream in the creaming mixture and, therefore, the amount of skimmilk culture to standardize the fat content. Cheese creamed with such a preparation has an appreciable amount of flavor immediately after creaming.

Dairy products manufactured with lactic culture commonly contain some biacetyl. Such products are butter, cultured buttermilk, cottage cheese, and cultured sour cream. A considerable amount of information has been published on methods for increasing the biacetyl content of butter (1) and cultured buttermilk (3, 9), but very little information of this type is available on cottage cheese and cultured sour cream.

Nelson and Brence (6) applied some of the methods known to increase volatile acid and biacetyl in lactic cultures to cottage cheese. However, cultures which contained considerable volatile acid did not yield cottage cheese with a high volatile acid content. No relationship was found between the flavor of a culture and the flavor of cottage cheese made with it. They noted that the addition of 0.008 to 0.05% citric acid to the cheese milk increased the volatile acid content of the resulting cheese and generally improved its flavor.

Mather and Babel (5) found that many samples of commercially creamed cottage cheese contained less than 0.5 p.p.m. biacetyl. The addition of citric acid to a creaming mixture for cottage cheese did not increase the biacetyl content

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when the cheese was held at 45° F. Addition of both citric acid and lactic culture to the creaming mixture increased the biacetyl content of the creamed cottage cheese when the holding temperature was 45° F., but a sour flavor was evident after several days of storage.

The object of this study was to develop a method for increasing and standardizing the biacetyl content of creamed cottage cheese.

METHODS

Biacetyl and acetylmethylcarbinol. The colorimetric method of Prill and Hammer (7) was used for the estimation of biacetyl and acetylmethylcarbinol in cottage cheese, creamed cottage cheese, and cultures. Color intensity was measured with a Beckman spectrophotometer (Model B) at a wave length of 430 mμ. Dimethylglyoximate was used in constructing the standard cure.

Volatile acid. A 50-g. sample was acidified with 3 ml. of 1.0 *N* sulfuric acid and steam-distilled. The first 100 ml. of distillate was titrated with 0.1 *N* sodium hydroxide, using phenolphthalein as indicator.

Determination of pH. Samples of cheese were prepared and tested by the method of Sanders (8). A Leeds and Northrup Type K potentiometer fitted with a quinhydrone electrode and saturated calomel cell was used for all pH measurements on cheese. A Beckman Laboratory Model pH meter was used to adjust the pH of cultures and creaming mixtures.

Cottage cheese. The long-set method outlined by Hales (4) was used for cottage cheese manufacture. When creamed, a 12% fat creaming mixture was added at the rate of one part creaming mixture to two parts cheese. The creamed cottage cheese was salted at the rate of 1%.

RESULTS

Effect of addition of citric acid and a culture of S. citrovorus to a creaming mixture on the biacetyl content of creamed cottage cheese. The rather low pH of well-mixed samples of creamed cottage cheese suggested that a pure culture of a citric acid–fermenting *Streptococcus,* of the type commonly associated with *Streptococcus lactis* in lactic cultures, might produce biacetyl from citric acid when added to a creaming mixture. The culture used in this trial was selected on the basis of its ability to produce biacetyl from citric acid at a relatively low pH.

One batch of cottage cheese was divided into five lots and treated as follows: (1) No treatment, (2) creamed and salted, (3) creamed, salted, and 0.15% citric acid added, (4) creamed, salted, and 1% of *S. citrovorus* culture added, (5) same as Lot 4 except that 0.15% citric acid was added. Samples of each lot were held at 45° F. and examined at intervals for biacetyl, acetylmethylcarbinol, and pH.

Table 1 shows that the biacetyl content of this batch of cottage cheese decreased slightly over a nine-day period; the carbinol content and pH remained about the same. Creaming the cottage cheese decreased the biacetyl content and a further decrease occurred during holding. The addition of 0.15% citric acid to creamed cottage cheese did not increase the biacetyl and carbinol contents, but

TABLE 1

Biacetyl, acetylmethylcarbinol, and pH values of cottage cheese and creamed cottage cheese without and with addition of citric acid and/or a culture of *Streptococcus citrovorus*

Product and treatment	Days held at 45° F.														
	0	2	4	6	9	0	2	4	6	9	0	2	4	6	9
	Biacetyl					Acetylmethylcarbinol					pH				
	(*p.p.m.*)					(*p.p.m.*)									
Cottage cheese	1.6	1.6	1.4	1.3	1.0	11.3	18.2	16.2	18.5	17.3	4.73	4.80	4.75	4.73	4.72
Creamed cottage cheese	1.1	1.0	1.0	0.9	0.6	11.5	18.8	8.3	7.7	7.5	4.90	5.03	5.03	5.00	4.98
Creamed cottage cheese plus citric acid	1.1	1.0	1.0	1.0	1.0	9.7	11.9	9.8	13.7	9.2	4.71	4.75	4.75	4.75	4.71
Creamed cottage cheese plus *S. citrovorus*	1.1	1.3	1.4	1.6	1.8	11.5	17.6	19.6	28.8	21.0	4.90	5.00	4.93	4.83	4.57
Creamed cottage cheese plus citric acid and *S. citrovorus*	1.1	1.3	1.7	2.3	2.7	9.4	17.0	27.3	41.8	63.7	4.68	4.72	4.73	4.73	4.59

seemed to stabilize the amounts initially present; such an addition gave a definite decrease in pH. Addition of *S. citrovorus* to creamed cottage cheese resulted in a progressive increase in biacetyl throughout the holding period. This addition also gave increases in carbinol for six days, a slight decrease occurring at nine days. The addition of citric acid and *S. citrovorus* culture to creamed cottage cheese gave progressive increases in biacetyl and carbinol throughout the holding period, and the increases were greater than those obtained without added citric acid.

Formation of biacetyl and acetylmethylcarbinol in a creaming mixture for cottage cheese. Since previous experiments indicated that biacetyl could be produced in creamed cottage cheese inoculated with *S. citrovorus*, but that some holding time was necessary, trials were conducted to determine whether biacetyl could be formed in the creaming mixture prior to its addition to cottage cheese. The creaming mixture used for these studies was heated in flowing steam for 30 min. and then cooled to 45° F.

Data presented in Table 2 show that a 12% fat creaming mixture with no additions (Lot 1), and held at 45° F., contained no biacetyl or carbinol during four days. Also, addition of a culture of *S. citrovorus* to the creaming mixture (Lot 2) did not increase the biacetyl or carbinol content at 45° F. The addition of *S. citrovorus* plus 0.15% citric acid to the creaming mixture (Lot 3) caused a very slight increase in both biacetyl and carbinol after holding three days at 45° F. A creaming mixture inoculated with *S. citrovorus* and held at 70° F. (Lot 4) did not contain biacetyl after holding for three days. However, when this lot was acidified with citric acid to pH 4.24 on the third day, a conspicuous increase in both biacetyl and carbinol occurred within 24 hr. at 70° F. Much smaller amounts of these compounds were formed when the acidified creaming mixture was held at 45° F. (Lot 5). No biacetyl was detected in a creaming

TABLE 2

Effect of holding temperature, pH, and citric acid on the production of biacetyl and acetylmethylcarbinol in a cottage cheese creaming mixture inoculated with *Streptococcus citrovorus*

Lot No.[a]	Storage temperature	Holding time *(days)*											
		Biacetyl				Acetylmethylcarbinol				pH			
		0	1	3	4	0	1	3	4	0	1	3	4
		(p.p.m.)				*(p.p.m.)*							
1	45	0.0	0.0	0.0	0.0	0.4	0.4	0.0	0.2	6.65	6.62	6.70	6.50
2	45	0.0	0.0	0.0	0.0	0.3	0.8	0.4	0.2	6.50	6.54	6.25	6.20
3	45	0.0	0.0	0.3	0.4	0.3	1.1	7.3	7.1	5.78	5.92	5.85	5.75
4	70	0.0	0.0	0.0	6.5	0.3	0.7	0.2	149.5	6.48	6.40	6.25	4.18
5	45				2.3				56.9				4.08
6	70	0.0	0.0	0.0	6.0	0.2	26.0	20.0	139.8	5.64	5.89	5.88	4.12
7	45				1.4				126.6				4.02

[a] Lot 1. Creaming mixture (12% fat); Lot 2. Creaming mixture plus *S. citrovorus*; Lot 3. Creaming mixture plus *S. citrovorus* and 0.15% citric acid; Lot 4. Creaming mixture plus *S. citrovorus;* acidified to pH 4.24 on third day with citric acid; Lot 5. Same as Lot 4, but held at 45° F. after acidifying to pH 4.24; Lot 6. Creaming mixture plus *S. citrovorus* and 0.15% citric acid; acidified to pH 4.22 on third day with citric acid; Lot 7. Same as Lot 6, but held at 45° F. after acidifying to pH 4.22.

mixture containing *S. citrovorus* plus 0.15% citric acid and held at 70° F. for three days (Lot 6), but when this lot was acidified with citric acid to pH 4.22 on the third day, a large increase in biacetyl and carbinol occurred. The increase was larger with holding at 70° F. (Lot 6) than at 45° F. (Lot 7).

Effect of an acidified creaming mixture containing S. citrovorus on the biacetyl content of creamed cottage cheese. A creaming mixture containing 12% fat was heated in flowing steam for 1 hr., cooled to 70° F., and inoculated with 4% of a culture of *S. citrovorus*. The mixture was held at 70° F. for 48 hr. and acidified with sterile citric acid solution (10%) to pH 4.38. After the pH adjustment, the mixture was held for 21 hr. at 70° F.

Table 3 gives the biacetyl, acetylmethylcarbinol, and pH values of cottage cheese (Lot 1), regularly creamed cottage cheese (Lot 2), and cottage cheese creamed with the special creaming mixture containing *S. citrovorus* (Lot 3). The cottage cheese and regularly creamed cottage cheese showed only small variations in biacetyl throughout a seven-day holding period at 45° F. Creaming cottage cheese with the regular creaming mixture decreased the biacetyl content and increased the pH. The cottage cheese creamed with the special creaming mixture contained 3.6 p.p.m. of biacetyl immediately after creaming and 5.7 p.p.m. after holding for seven days at 45° F. The carbinol content of this lot also was considerably greater than either of the two other lots. Regularly creamed cottage cheese had the highest pH and the uncreamed product the lowest pH.

Production of biacetyl, acetylmethylcarbinol, and volatile acid in a skimmilk culture of S. citrovorus adjusted to various pH levels with citric acid. In the experiments which follow, skimmilk is used as the growth medium for *S. citrovorus* and also for the development of fermentation products from citric acid. Therefore, an experiment was conducted to determine the most favorable pH for the production of biacetyl and volatile acid, compounds which contribute to the flavor of creamed cottage cheese.

A large lot of skimmilk was heated in flowing steam for 1 hr. and cooled to 70° F. One portion was removed to serve as a control. The remainder was inoculated with 2% of a culture of *S. citrovorus*, divided into seven portions, and incubated at 70° F. for 48 hr. After incubation, sterile citric acid solution (15%) was added in varying amounts to give a pH range of 5.97 to 3.72. The acidified samples were held at 70° F. for 24 hr. and analyzed for biacetyl, acetylmethylcarbinol, and volatile acid. Results of these analyses are given in Table 4.

Only small amounts of biacetyl were presented in the skimmilk samples having a pH above 5.01. The difference between pH 5.01 and 5.50 was striking, since the former yielded about a fivefold increase over the latter. Further increases occurred as the pH was decreased, but the amount of increase was not great at pH values less than 4.31. Acetylmethylcarbinol and volatile acid increased as the pH was decreased and maximum amounts were obtained at pH 4.31. Further lowering of the pH resulted in slightly smaller amounts of these compounds.

Preparation of a creaming mixture for standardizing the biacetyl content of creamed cottage cheese. The development of biacetyl, acetylmethylcarbinol, and

TABLE 3

Biacetyl, acetylmethylcarbinol, and pH values of cottage cheese and creamed cottage cheese, using a regular creaming mixture and a creaming mixture inoculated with *Streptococcus citrovorus* and adjusted to pH 4.38 with citric acid

Lot No.[a]	Days held at 45° F.														
	0	2	4	6	7	0	2	4	6	7	0	2	4	6	7
	Biacetyl (p.p.m.)					Acetylmethylcarbinol (p.p.m.)					pH				
1	0.9	1.2	1.1	1.2	0.9	9.7	2.9	7.5	9.9	15.9	4.67	4.60	4.60	4.60	4.60
2	0.6	0.7	0.6	0.7	0.6	9.3	10.7	9.9	9.6	8.2	5.06	4.99	4.98	4.99	5.00
3	3.6	4.7	4.8	5.0	5.7	84.6	97.3	102.0	114.0	128.3	4.62	4.78	4.77	4.75	4.80

[a] Lot 1. Cottage cheese; Lot 2. Cottage cheese creamed with regular creaming mixture (12% fat); Lot 3. Cottage cheese creamed with a special creaming mixture containing *S. citrovorus* and adjusted to pH 4.38 with citric acid.

TABLE 4

Biacetyl, acetylmethylcarbinol, and volatile acids content of a skimmilk culture of *Streptococcus citrovorus* adjusted to various pH levels with citric acid

pH	Biacetyl	Acetylmethylcarbinol	Volatile acids
	(p.p.m.)	*(p_4p.m.)*	*(ml. N/10 NaOH)*
6.41[a]	0.2	0.8	0.2
5.97	0.4	1.6	1.2
5.50	0.7	2.1	1.3
5.01	3.4	49.6	2.1
4.62	6.1	119.3	2.4
4.31	10.7	274.1	3.1
4.02	11.1	230.1	2.9
3.72	12.0	222.0	2.5

[a] No pH adjustment. No citric acid added.

volatile acid in a 12% fat creaming mixture presented certain problems, particularly with regard to standardization of the biacetyl content. Therefore, the following method was adopted.

Skimmilk is heated to 190° F. and held for 1 hr., or it can be heated in an autoclave at 250° F. for 10 min. After cooling to 70° F., it is inoculated with 2 to 4% of a culture of *S. citrovorus* and incubated at 70° F. for about 24 hr. Sterile citric acid solution (15%) is added to lower the pH to 4.3, and the material is further incubated at 70° F. for 20 to 24 hr. Considerable biacetyl, acetylmethylcarbinol, and volatile acid is formed. The skimmilk thus prepared is used to adjust the fat content of cream to about 12%. The mixture is homogenized at 500 to 1,000 p.s.i. Standardization of biacetyl is accomplished by varying the fat content of cream used in the creaming mixture and, therefore, the amount of skimmilk culture to standardize the fat content. When 20% fat cream is used for preparing a mixture containing 12% fat, the ratio of cream to fermented skimmilk is 1.5 to 1.0, by weight.

Table 5 gives the biacetyl, acetylmethylcarbinol, and pH values of a batch of cottage cheese treated as follows: (1) No treatment, (2) creamed with a regular creaming mixture containing 12% fat, (3) creamed with a mixture made up of 60 parts 20% cream and 40 parts of the specially treated skimmilk containing *S. citrovorus* (when added to cheese, the specially treated skimmilk culture constituted 13.5% of the creamed cottage cheese), and (4) creamed with a creaming mixture made up of 80 parts cream and milk containing 15% fat and 20 parts of the specially treated skimmilk containing *S. citrovorus* (when added to cheese, the specially treated skimmilk culture constituted 6.75% of the creamed cottage cheese).

The cottage cheese used in this experiment contained about 0.5 p.p.m. biacetyl initially and it remained at this level throughout an 11-day holding period at 45° F. Creaming with a regular creaming mixture decreased the biacetyl content to 0.3 p.p.m. When creamed with the special creaming mixture so that the *S. citrovorus* culture constituted 13.5% of the creamed cottage cheese, the biacetyl content was 1.6 p.p.m. initially and it increased to 2.8 p.p.m. during 11 days of storage at 45° F. The lot in which *S. citrovorus* culture constituted

TABLE 5

Biacetyl, acetylmethylcarbinol, and pH values of cottage cheese, and creamed cottage cheese using a regular and special creaming mixture

Product	Days held at 45° F.														
	0	4	6	8	11	0	4	6	8	11	0	4	6	8	11
	Biacetyl (p.p.m.)					Acetylmethylcarbinol (p.p.m.)					pH				
Cottage cheese	0.5	0.6	0.5	0.5	0.5	8.6	10.0	8.3	9.0	8.6	4.68	4.66	4.68	4.69	4.65
Regularly creamed cottage cheese	0.3	0.3	0.3	0.4	0.3	6.2	6.4	7.1	6.4	6.0	4.99	5.05	5.05	5.02	5.03
Specially creamed cottage cheese[a]	1.6	2.2	2.2	2.4	2.8	39.6	52.2	43.4	51.2	50.6	4.94	5.00	4.98	4.98	4.92
Specially creamed cottage cheese[b]	0.9	1.4	1.4	1.7	1.9	22.3	24.8	27.1	28.9	35.1	5.01	5.00	4.98	4.97	5.00

[a] *Streptococcus citrovorus* culture constituted 13.5% of the creamed cottage cheese.
[b] *Streptococcus citrovorus* culture constituted 6.75% of the creamed cottage cheese.

6.75% of the creamed cottage cheese had a biacetyl content of 0.9 p.p.m. initially and it increased to 1.9 p.p.m. during 11 days of storage at 45° F. The largest amount of acidified *S. citrovorus* culture decreased the pH of the creamed cottage cheese by 0.04 to 0.11 of a pH unit.

This experiment was repeated in another trial, in which the cottage cheese and creamed cottage cheese were held at 50° F. The data obtained are reported in Table 6.

The cottage cheese used in this trial contained 0.3 p.p.m. of biacetyl initially and only slight variations were noted during the eight-day holding period. Also, the acetylmethylcarbinol content and pH varied only slightly. The cottage cheese had a bitter and yeasty flavor at eight days. The regularly creamed cottage cheese contained 0.2 to 0.3 p.p.m. biacetyl throughout the holding period. The carbinol content and pH remained quite stationary for five days, but decreased considerably at eight days. This lot also had a bitter and yeasty flavor at eight days. Cottage cheese creamed with the largest amount of acidified *S. citrovorus* culture had an initial biacetyl content of 1.4 p.p.m., which increased to 2.6 p.p.m. in five days, followed by a decrease to 1.8 p.p.m. in eight days. The carbinol content followed the same general trend as the biacetyl content and the pH remained quite constant throughout the holding period. This lot did not develop an off-flavor within eight days. Cottage cheese creamed with the smaller amount of acidified *S. citrovorus* culture showed the same general trend in biacetyl and carbinol as the lot creamed with the larger amount. However, a decrease in pH was noted at eight days and at this time the cheese had a bitter and yeasty flavor.

DISCUSSION

In previous experiments (5), the addition of lactic culture to a creaming mixture resulted in the formation of some biacetyl, but gave the cheese a sour flavor after holding at 45° F. Therefore, the use of a culture of *S. citrovorus* seemed to offer some possibilities. *S. citrovorus* does not form lactic acid from lactose but does produce biacetyl from citric acid in pure culture at a favorable pH.

The addition of *S. citrovorus* to a creaming mixture for cottage cheese caused a definite increase in biacetyl (Table 1), but approximately nine days at 45° F. was required to obtain a desired level of flavor. Acetylmethylcarbinol, the precursor of biacetyl, began to decrease at nine days. The addition of 0.15% citric acid to the creamed cottage cheese, plus *S. citrovorus*, resulted in a desired flavor level being attained in about four days. The carbinol content of this lot increased throughout the nine-day holding period.

The time required to attain a desired level of flavor when *S. citrovorus* and citric acid were added to creamed cottage cheese was considered a definite disadvantage. However, the results suggested that it might be possible to develop the flavor compounds (biacetyl and volatile acid) in a cottage cheese creaming mixture before its addition to cottage cheese.

Little or no biacetyl was formed in a creaming mixture containing *S. citrovorus* or *S. citrovorus* plus 0.15% citric acid and held four days at 45° F. (Table 2).

This temperature was unfavorable for the growth of *S. citrovorus* and the pH of the creaming mixture was too high for citric acid fermentation. Although 70° F. was a satisfactory growth temperature for *S. citrovorus*, no biacetyl was formed by the organism at this temperature until the pH of the creaming mixture was lowered. Decreasing the pH to 4.24 with citric acid resulted in the accumulation of much biacetyl (6.5 p.p.m.) in 24 hr. When 0.15% citric acid was added to the creaming mixture before the addition of *S. citrovorus*, followed by incubation and acidification with citric acid to pH 4.22, slightly less biacetyl was produced. This decrease was probably due to less growth of *S. citrovorus* because of the lower initial pH.

A creaming mixture inoculated with *S. citrovorus*, incubated 48 hr. at 70° F., then acidified with citric acid to pH 4.38 and further incubated for 21 hr. at 70° F., contained about 9 p.p.m. of biacetyl. This creaming mixture, added to cottage cheese containing 0.9 p.p.m. biacetyl, increased the biacetyl content to 3.6 p.p.m. (Table 3). Such an addition resulted in the creamed cottage cheese having considerable flavor immediately after creaming. Additional flavor was formed in the creamed cottage cheese as it was held at 45° F. Cottage cheese creamed in this manner has a lower pH than regularly creamed cottage cheese, but the product did not taste sour because of the masking effect caused by biacetyl and volatile acid.

Heating a creaming mixture containing 12% fat in flowing steam for 1 hr., or in an autoclave at 250° F. for 10 min., caused some fat separation. Acidification of the creaming mixture with citric acid caused precipitation of the casein. Although homogenization partially overcame these physical changes, a creaming mixture undergoing such treatment gave a creamed cottage cheese that was quite dry in appearance, and in texture when eaten. To eliminate these undesirable features, skimmilk was used as the growth medium for *S. citrovorus* and was later acidified with citric acid. With this procedure, the flavor compounds were formed in skimmilk by a pure culture of *S. citrovorus* and the skimmilk was mixed with cream and homogenized to form the creaming mixture. This method eliminated the physical defects noted earlier and also permitted some standardization of the biacetyl content, since the amount of skimmilk culture used could be varied by the use of cream with different fat contents.

A 20% fat cream standardized to 12% with the acidified skimmilk culture of *S. citrovorus* yielded creamed cottage cheese with a pleasing flavor and a fairly high initial biacetyl content (Table 5). The biacetyl content of cottage cheese creamed in this manner also increased during an 11-day holding period at 45° F.

The storage temperature of cottage cheese has considerable effect on the time required to produce certain bacteriological defects (2). Trials were made to determine what effect a storage temperature of 50° F. would have on the biacetyl content of cottage cheese creamed with the special creaming mixture containing *S. citrovorus*. A representative trial reported in Table 6 shows that the biacetyl content increases for about five days at 50° F. and then decreases. It should be noted that flavor defects were evident sooner in cottage cheese, regularly creamed

TABLE 6

Biacetyl, acetylmethylcarbinol, and pH values of cottage cheese, regularly creamed cottage cheese, and specially creamed cottage cheese held at 50° F.

Product	Days held at 50° F.											
	0	2	5	8	0	2	5	8	0	2	5	8
	Biacetyl				Acetylmethylcarbinol				pH			
	(p.p.m.)				(p.p.m.)							
Cottage cheese	0.3	0.4	0.4	0.2*	8.7	8.6	8.4	8.0	4.61	4.58	4.63	4.55
Regularly creamed cottage cheese	0.2	0.2	0.3	0.2*	6.5	7.0	6.1	0.7	4.97	4.96	4.95	4.86
Specially creamed cottage cheese[a]	1.4	2.1	2.6	1.8	43.4	53.9	63.4	46.2	4.87	4.88	4.85	4.86
Specially creamed cottage cheese[b]	0.9	1.4	1.7	1.3*	27.1	31.0	37.5	22.7	4.93	4.93	4.93	4.88

*A bitter and yeasty flavor was noted at this time.

[a] *Streptococcus citrovorus* culture constituted 13.5% of the creamed cottage cheese.

[b] *Streptococcus citrovorus* culture constituted 6.75% of the creamed cottage cheese.

cottage cheese, and cottage cheese creamed with a small amount of *S. citrovorus* culture, than in cottage cheese creamed with a larger amount of this culture.

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EFFECT OF THERMAL OXIDATIVE POLYMERIZATION ON THE GROWTH-PROMOTING VALUE OF SOME FRACTIONS OF BUTTERFAT[1]

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SUMMARY

Butterfat was fractionated into acetone or alcohol-soluble and -insoluble fractions at 0 and 20° C., respectively. The butterfat and the four fractions were thermally oxidized at 200° C. for 24 hr., to study the effect of heat on the growth-promoting value of the fractions when fed to rats at a 20% level, in a diet composed of 31% casein, 44% cerelose, 4% Wesson salts, and supplemented with fat- and water-soluble vitamins. The results indicated that thermally oxidized butterfat or the thermally oxidized acetone-insoluble fraction of butter did not exhibit any differences in the growth of rats when compared with those fed fresh fats. When rats were fed corn oil or hydrogenated soybean oil thermally oxidized at 200° C. for 24 hr., they gained significantly less weight than did those fed the fresh fats. When these oils were mixed with 30% of the acetone-insoluble butterfat and thermally oxidized at 200° C. for 24 hr. and fed to rats, no differences in growth were noted. These data indicate that butterfat contains triglycerides which are acetone-insoluble and which are able to counteract the effect of toxic products or prevent the formation of toxic products during the thermal polymerization of a fat.

A number of workers have shown that heat-polymerized oils and fats have less nutritive value than the corresponding fresh oils and fats (2, 3, 7). The growth-depressing effect of such fat has been attributed to the formation of toxic materials during heat polymerization. Johnson *et al.* (4) have, however, reported that thermally polymerized butterfat did not produce growth-depressing effects in rats. It was, therefore, possible that butterfat might contain some factor or triglyceride fraction which prevented the formation of toxic materials or was relatively stable to heat polymerization. In the present study, butterfat was fractionated from absolute ethanol or acetone and the effect of heat polymerization on the growth-promoting value of these fractions determined.

EXPERIMENTAL PROCEDURE

Fractionation. Butter[2] was heated to 50° C. in a hot water bath and the butterfat separated by removing the lower aqueous layer in a separatory funnel. The butterfat was washed three times with hot (60° C.) distilled water and dehydrated by shaking with anhydrous sodium sulfate for about 10 min. The clear butterfat obtained by filtering through cotton wool was subsequently used for the fractionation procedure. Butterfat[2] was fractionated into an alcohol-soluble and an alcohol-insoluble fraction by dissolving 1-kilo lots of the fat in 10 liters of hot (70° C.) absolute ethyl alcohol; the solution was cooled to 20° C.

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[1] Funds for these studies were supplied by the American Dairy Association.
[2] Churned at the University Creamery through the courtesy of Dr. S. L. Tuckey.

and kept at this temperature overnight. The insoluble fraction was removed by filtration and both fractions freed of the solvent on a steam bath under vacuum. Butterfat was also fractionated into an acetone-soluble and an acetone-insoluble fraction by dissolving 1-kilo lots of the fat in 10 liters of hot (50° C.) acetone; the solution was cooled to 0° C. and kept at this temperature overnight. The insoluble fraction was removed by filtration and both fractions freed of the solvent on a steam bath with the aid of a Rinco evaporator under vacuum. Fractionation of butterfat from ethyl alcohol gave 25% of an alcohol-insoluble fraction, while fractionation of butterfat from acetone yielded 30% of an acetone-insoluble fraction. The refractive index and the iodine value were higher for the acetone-soluble fraction than for the three other fractions (Table 1).

TABLE 1

Iodine value and fatty acid composition of butterfat and its various fractions

Sample	Ref. index at 40° C.	Fatty acids (mole %)				Iodine value	
		Butyric	Oleic	Linoleic	Arachidonic	Before thermal oxidation	After thermal oxidation
1. Butterfat	1.4542	11.2	28.8	2.1	0.2	38	28
2. Acetone-soluble butterfat	1.4551	13.1	38.9	2.8	0.2	53	33
3. Acetone-insoluble butterfat	1.4539	6.8	14.6	0.0	0.0	25	22
4. Alcohol-soluble butterfat	1.4538	14.1	30.6	2.4	0.2	40	28
5. Alcohol-insoluble butterfat	1.4544	3.0	17.9	0.9	0.0	30	16

The butyric acid content of the four fractions was determined by the method of Keeney (8), and the unsaturated fatty acids were determined with the aid of a spectrophotometric method (9). The results indicated that the butyric acid content of the acetone-soluble or of the alcohol-soluble was significantly higher than that of the corresponding insoluble fraction. The total amount of oleic and linoleic acids in the alcohol-soluble fraction was slightly lower than that present in the acetone-soluble fraction, and the total amount of the unsaturated fatty acids in the alcohol-insoluble fraction was significantly lower than that in the original butterfat.

The whole butterfat and the four fractions were further analyzed for trisaturated glyceride (GS_3) content, according to the method outlined by Kartha (5). From the amount of trisaturated glycerides present, and the total saturated fatty acids in each fraction, the triglyceride composition of butterfat and the four fractions were calculated (Table 2) according to Kartha's method (6). The acetone-insoluble fraction did not contain any triunsaturated glycerides (GU_3) or GSU_2; whereas, the alcohol-insoluble fraction contained 2% monosaturated-diunsaturated glycerides (GSU_2) and no GU_3. The amount of GS_3 in the acetone-insoluble and the alcohol-insoluble fractions was found to be more than twice that present in the corresponding soluble fractions. The amount of

TABLE 2
Comparison of triglyceride composition of butterfat and its fractions from acetone and alcohol (molar %)

Fat	GS_3	GS_2U	GSU_2	GSU_3
Butterfat	32.0	45.6	20.3	2.1
Acetone-soluble butterfat	24.0	47.7	24.1	4.2
Acetone-insoluble butterfat	56.2	43.8	0.0	0.0
Alcohol-soluble butterfat	26.2	49.5	21.0	3.2
Alcohol-insoluble butterfat	52.5	45.5	2.0	0.0

disaturated-monounsaturated glycerides (GS_2U) was found to be almost the same in all the fractions.

Nutrition. To study the effect of heat on their growth-promoting value, the four fractions were thermally oxidized according to the method of Johnson *et al.* (4). The samples were heated in stainless steel beakers at 200° C. for 24 hr. There was generally a reduction in the iodine value; a maximum reduction of 20 occurred in the acetone-soluble fraction (Table 1).

A modified synthetic diet which contained 44% cerelose, 31% casein, 4% Wesson salts (10), and 20% of the various fat fractions was used in all of the feeding trials. The diet was supplemented with 2 g. of a water-soluble vitamin mixture per kilogram of food. This mixture was composed of choline chloride 93.5 mg., thiamin hydrochloride 1.24 mg., riboflavin 1.24 mg., pyridoxine hydrochloride 1.24 mg., calcium pantothenate 2.48 mg., folic acid 0.30 mg., and 1.9 g. of glucose. One drop per rat of the following fat-soluble vitamin mixture was administered once each week: 5 g. vitamin A (200,000 U.S.P. units, courtesy of Distillation Products), 0.0054 g. vitamin D_2 and 2.535 g. vitamin E (mixed tocopherols) in 100 ml. of olive oil. Six male rats were used in each group and fed the various diets on an equalized restricted feed intake basis for a period of 4 wk.

Whether the acetone-insoluble fraction contained a growth-promoting factor or factors which could protect the triglycerides against the formation of toxic substances was studied as follows: hydrogenated soybean oil, corn oil, or the acetone-soluble fraction of butterfat was mixed with 30% of the acetone-insoluble fraction of butterfat, and the various mixtures heated at 200° for 24 hr. The various mixtures of these fats were fed to rats for a 1-wk. period at a 20% level, and their effect on growth noted. The shorter test period was necessary, since animals on thermally oxidized corn oil or hydrogenated soybean oil did not live more than ten to 14 days under conditions of restricted feeding.

RESULTS AND DISCUSSION

The animals which had been fed the thermally oxidized butterfat or the thermally oxidized acetone-insoluble fraction did not exhibit any differences in growth when compared with those fed fresh fats (Table 3). A slight depression in growth was noted in the animals which had received the thermally oxidized alcohol-soluble or alcohol-insoluble fractions of butterfat when compared with

TABLE 3

Effect of feeding fresh and thermally oxidized butterfat and its various fractions at 20% level on the growth of rats

No.	Fat	Treatment	Average initial weight of rats	Average gain[a] in weight of rats (in 4 wk.)	Significance
			(g.)	*(g.)*	
1.	Butterfat	None	45.4	46.8 ± 1.0	N.S.
2.	Butterfat	T.O.	44.0	44.4 ± 1.6	
3.	Alcohol-soluble butterfat	None	45.4	49.2 ± 1.0	S
4.	Alcohol-soluble butterfat	T.O.	45.2	39.8 ± 1.8	
5.	Alcohol-insoluble butterfat	None	45.7	41.2 ± 2.5	S
6.	Alcohol-insoluble butterfat	T.O.	45.0	31.5 ± 1.6	
7.	Acetone-soluble butterfat	None	47.0	53.5 ± 2.4	S
8.	Acetone-soluble butterfat	T.O.	44.8	35.7 ± 2.7	
9.	Acetone-insoluble butterfat	None	47.6	40.0 ± 1.2	N.S.
10.	Acetone-insoluble butterfat	T.O.	44.6	38.4 ± 1.5	

[a] ± Standard error of mean.
S. Differences in weight statistically significant at 1% level.
N.S. Differences in weight statistically not significant at 1% level.
T.O. Thermally oxidized at 200° C. for 24 hr.

fresh samples. However, a marked depression in growth was noted on diets which contained the thermally oxidized acetone-soluble fraction of butterfat.

It was noted that the animals which had received thermally oxidized hydrogenated soybean oil or thermally oxidized corn oil gained significantly less weight than those on fresh fats (Table 4). However, when these fats were mixed with 30% of the acetone-insoluble fraction from butterfat before thermal oxidation, soybean and corn oil seemed to be more stable to thermal oxidation. There were practically no differences in the growth of rats which had received fresh or

TABLE 4

Effect of feeding fresh and thermally oxidized mixtures of fats at 20% level on the growth of rats

No.	Fat mixture	Treatment	Average initial weight of rats	Average gain[a] in weight of rats (1 wk)	Significance
			(g.)	*(g.)*	
1.	Fat Mixture A	None	58.6	23.0 ± 1.7	N.S.
2.	Fat Mixture A	T.O.	58.0	21.8 ± 1.4	
3.	Hydrogenated soybean oil	None	57.6	28.8 ± 1.7	S
4.	Hydrogenated soybean oil	T.O.	58.4	20.6 ± 1.1	
5.	Fat Mixture B	None	58.0	25.4 ± 1.5	N.S.
6.	Fat Mixture B	T.O.	57.2	25.8 ± 1.8	
7.	Corn oil	None	57.6	30.2 ± 1.9	S
8.	Corn oil	T.O.	58.2	21.8 ± 1.6	
9.	Fat Mixture C	None	53.0	32.0 ± 0.6	N.S.
10.	Fat Mixture C	T.O.	53.2	31.4 ± 1.3	

[a] ± Standard error of mean.
S. Difference in weight statistically significant at 1% level.
N.S. Differences in weight statistically not significant at 1% level.
T.O. Thermally oxidized at 200° C. for 24 hr.
Fat Mixture A—Acetone-soluble butterfat 70% + acetone-insoluble butterfat 30%.
Fat Mixture B—Hydrogenated soybean oil 70% + acetone-insoluble butterfat 30%.
Fat Mixture C—Corn oil 70% + acetone-insoluble butterfat 30%.

thermally oxidized fats when the latter contained the acetone-insoluble fraction of butterfat.

It is possible that the thermal stability of butterfat may be due to one or more factors. The degree of unsaturation does not seem to be the only factor involved in thermal polymerization. If it were, whole butterfat which had the same iodine value as the alcohol-soluble butterfat and a higher iodine value than the alcohol-insoluble fraction of butterfat, should have shown growth depression after heat treatment. Similarly, hydrogenated soybean oil and corn oil, even after the addition of 30% of the acetone-insoluble fraction of butterfat, had a fairly high iodine value; namely, 49 and 82, respectively. However, these samples did not depress growth in rats on heat treatment, which indicated that the heat stability of these fats was probably dependent upon the presence of relatively stable triglycerides.

Butterfat seemed to contain specific triglycerides, which are acetone-insoluble and which are able to counteract the effects of toxic products or prevent the formation of toxic products during heating. This was evident from the fact that butterfat, from which the acetone-insoluble fraction had been removed by crystallization from acetone at 0° C. and subjected to thermal oxidation, produced growth depression in rats. The growth-depressing effects produced by thermal oxidation did not appear, however, when this fraction was added back to the acetone-soluble fraction before heating it to 200° C. for 24 hr. There were no differences in the growth of rats fed diets containing this mixture when it was either fresh or thermally oxidized. The specificity of the acetone-insoluble fraction to thermal stability is further confirmed by the fact that corn oil or hydrogenated soybean oil did not depress growth in rats when it was mixed with approximately 30% of the acetone-insoluble fraction of butterfat prior to thermal oxidation.

The triglyceride composition of butterfat and the acetone-soluble and acetone-insoluble fractions of butterfat (Table 2) seemed to indicate that the specific triglycerides responsible for preventing growth depression are present in the trisaturated glycerides. However, it was noted that the alcohol-insoluble fraction of butterfat, which contained a fairly high amount of trisaturated glycerides, depressed growth in rats after heat treatment. It may be presumed from this observation that these specific acetone-insoluble trisaturated glycerides were disproportionately distributed in both the alcohol-soluble and alcohol-insoluble fractions. The triglycerides were, thus, not in the right proportion in either of the two fractions and were not able to prevent formation during heat polymerization of substances responsible for growth depression.

It is not possible to state from the results of the present experiments how the specific acetone-insoluble glycerides of butterfat prevented the formation of toxic products during heat treatment. It is known that molecular rearrangement of a triglyceride mixture occurs at an appreciable rate at about 275° C. when no catalyst is present (1). The fats in the present experiments were heated at 200° C. for 24 hr. Though there is no experimental evidence, it is a plausible assumption that the unsaturated fatty acids in the glyceride molecules might have

rearranged with the fatty acids in the specific acetone-insoluble glycerides in such a way that the formation of toxic polymeric materials was prevented to a certain degree. The molecular rearrangement of these triglyceride mixtures may be responsible to a certain extent for their heat stability.

The growth-promoting value of the heated fats may further be correlated with the reduction of their unsaturation after thermal oxidation. The acetone-soluble fraction of butterfat which showed a maximum depression in iodine value on heating also showed a maximum difference in the rate of growth between the rats in the control and the experimental groups. On the other hand, the acetone-insoluble fraction of butterfat, which showed the least depression in iodine value on heating, induced the least depression in growth.

CONCLUSIONS

Butterfat and the fractions of butterfat obtained from acetone and absolute ethanol were examined for their growth-promoting value after subjecting them to thermal polymerization at 200° C. for 24 hr. The acetone-insoluble fraction of butterfat seemed to contain a specific triglyceride which counteracted the growth-depressing effect of thermally polymerized corn or hydrogenated soybean oil.

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STUDIES OF THE EFFECTS OF DIETARY SODIUM FLUORIDE ON DAIRY COWS. V. A THREE-YEAR STUDY ON MATURE ANIMALS [1]

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SUMMARY

The effects of 30 and 50 p.p.m. F fed as the NaF salt to mature, adequately nurtured dairy cows for a period of three lactations have been studied. The fluorine concentration in the bones, urine and, to some extent, the soft tissues was increased. At both levels of intake some evidence of exostosis was evident in the excised long bones after they had been cleaned of adhering tissue. This condition was not discernible before slaughter, nor did it affect the gait of the animals. There was no evidence of impairment of lactation or effect on the maintenance of body weight. In these cows the incisor teeth were not clinically affected by fluorine ingestion. From these results, it would appear that lactating dairy cows first exposed at from 4 to 6 yr. of age will tolerate the ingestion of as much as 50 p.p.m. F for as long as three lactations, with no adverse effects other than mild exostosis of the long bones and increased concentrations of fluorine in the body.

The problem of chronic fluorine toxicosis in dairy cows has been studied experimentally, and cases have been reported in the field by many workers. The syndrome may be characterized by: mottling of the teeth; increased concentration of fluorine in the urine, skeleton, and teeth; exostosis and ankylosis of the bones; lameness and stiffness; excessive weight loss during lactation; and an impairment of lactation concurrent with an appetite depression [Phillips *et al.* (6)]. The severity of the above symptoms, if present at all, will depend upon the level and time of exposure to increased fluorine ingestion, and upon the age of the animal when first exposed. Recent studies at this (9, 10, 11) and other (4, 5, 8) experiment stations have yielded much information upon the tolerance of dairy cows first exposed to elevated dietary fluorine levels at 2 yr. of age. The objective of this experiment (809B) was to determine the effect of increased dietary fluorine (as NaF) upon mature dairy cows, and to compare the results with those obtained in previous studies.

EXPERIMENTAL PROCEDURE

Twelve mature Holstein cows (two–four complete lactations) were assigned to three groups of four each, according to milk production, size, and general appearance. The basal ration fed all cows consisted of 30–40 lb. corn silage, 15–24

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lb. alfalfa, brome, and clover hay, and 1 lb. of the grain mix per 4 lb. of milk produced. The grain mixture contained the following poundages: ground oats, 700; ground corn, 700; wheat bran, 400; linseed oil meal, 200; trace mineralized salt, 20; and irradiated yeast, 0.5. The ration contained from 3–5 p.p.m. of fluorine (9). Lot I received the basal ration, Lot II the basal plus 30 p.p.m. fluorine, and Lot III the basal plus 50 p.p.m. fluorine in the form of NaF.

The cattle were turned out for exercise daily but were never allowed on pasture. The fluorine was administered as a solution of NaF poured over the morning and night feed of grain and silage. The amount of fluorine given was adjusted weekly so that the necessary concentration on a dry weight basis was maintained. The cows were weighed monthly, were observed daily for signs of estrus, and were bred artificially.

Unless otherwise indicated, all fluorine analyses reported were made by the Alcoa Research Laboratory modification (1) of the Willard and Winter method (12).

RESULTS

As in the previous studies, the chief health problem during these studies was mastitis. There was no correlation between the incidence of chronic mastitis and fluorine intake. It was anticipated that the animals would be removed from experiment after completing at least 6 mo. of the third lactation. Two cows were discarded during the course of the experiment. Cow 28 (Lot I) was slaughtered after 3 mo. of its third lactation, due to severe mastitis, and Cow 34 (Lot III) was removed after two lactations because of difficulty in settling with calf.

Inspection of Table 1 reveals that there was no consistent adverse effect of added dietary fluorine upon milk production at the levels fed. The production observed was in the range that might be expected for animals of this age, breeding, and under these management practices. The records were simliar to those obtained in the previous studies (10). In all lots, the cows dropped in production upon entering the experimental herd. The management practice of confinement for all but a short time each day may, in part, explain these results.

The data presented in Table 2 indicate that these levels of dietary fluoride had no effect upon the maintenance of adult body weight, or in the variation of weight during each lactation.

Pathologic conditions of the bones have been observed and described in fluorine toxicosis (6, 7, 11). In this experiment, there were no palpatable exostoses while the animals were on experiment. However, after a thorough cleaning, the bones excised from cows receiving 30 p.p.m. F exhibited exostosis of periosteal origin, distributed as a thin mantle over the surface. Those fed 50 p.p.m. were quite rough and thickened, especially on the distal ends of the metacarpus and metatarsus. In the worst cases, periarticular structures and adjacent ligamentous attachments were calcified. The data are summarized in Table 3 and an arbitrary rating given each cow. It is obvious that when mature cows were fed NaF continuously for a period of 3 yr., exostosis occurred in the cows fed 50 p.p.m. F and, to some extent, in those of Lot II (30 p.p.m. F).

TABLE 1

Effect of the level of dietary fluoride (NaF) on milk production

Cow	Lot and p.p.m. F	Av. of previous lactations			1st lactation			2nd lactation			3rd lactation		
		Milk	Fat	Days	Milk	Fat	Days	Milk	Fat	Days	Milk	Fat	Days
25		.....	.....	.....	9,059	369	296	9,308	393	311	8,171[c]	278	262
26	I	.....	.....	.....	12,043	392	293	11,190	381	365	12,436	413	330
27	0	11,095(4)[a]	338	307	12,156	355	319	12,047	357	314	11,373	359	312
28		10,000(1)	420	.....	3,890	155	72[b]	5,658	207	301	2,601[c]	94	90
Av.					9,162	318	245	9,553	329	322	11,904[d]	386	321
29	II	10,063(2)	417	406	10,013	402	272	7,971	278	259	9,203	343	312
30	30	.....	.....	.....	8,343	296	309	10,851	381	321	9,135	309	275
31		11,745(4)	369	309	12,886	396	349	12,944	396	384	10,222	308	305
32		11,190(1)	445	316	11,021	340	282	9,598	251	304	10,532	312	325
Av.					10,565	359	303	10,314	327	317	9,773	318	304
33	III	14,136(2)	512	373	7,490	231	274	7,711	225	303	6,843	230	320
34	50	.....	.....	.....	9,566	314	341	11,040	369	385	.....[e]	.....	.....
35		12,240(1)	400	409	10,472	321	320	11,967	377	367	8,557	257	309
36		11,819(2)	415	271	8,583	274	273	11,220	394	243	11,711	427	287
Av.					9,028	285	302	10,485	341	325	9,037	305	305

[a] ( ) Number of lactations entering into this average; complete information not available on all cows.
[b] Acute mastitis, out of production early.
[c] Slaughtered during third lactation.
[d] Completed lactations only.
[e] Slaughtered previous to third lactation.

TABLE 2

Effect of level of dietary fluoride (NaF) upon body weight

Lot F added		1st lactation	2nd lactation	3rd lactation
(p.p.m.)				
I	Min.	1,194 ± 149[a]	1,245 ± 154[a]	1,327 ± 121[a]
Control	Max.	1,587 ± 133	1,549 ± 132	1,649 ± 102
	Av.	1,361 ± 130	1,382 ± 142	1,438 ± 144
II	Min.	1,239 ± 89	1,279 ± 97	1,325 ± 79
30 p.p.m. F	Max.	1,606 ± 135	1,662 ± 120	1,681 ± 95
	Av.	1,367 ± 107	1,432 ± 111	1,445 ± 79
III	Min.	1,174 ± 136	1,228 ± 124	1,192 ± 122
50 p.p.m. F	Max.	1,507 ± 231	1,562 ± 201	1,596 ± 162
	Av.	1,297 ± 164	1,362 ± 142	1,363 ± 115

[a] Standard deviation.

TABLE 3

Effect of level of dietary fluoride (NaF) upon urinary F concentration and over growth of bone

			Degree of effect on cleaned bone[b]	
Lot F added	Cow	F[a] in urine	Metacarpus	Metatarsus
(p.p.m.)		*(p.p.m.)*		
I				
0	25	3.8	0	0
(Control)	26	3.8	0	0
	27	3.8	1	1
	28	3.7	0	0
II	29	19.8	1	2
30 p.p.m. F	30	25.0	1	3
	31	25.0	1	3
	32	22.9	1	1
III	33	35.3	3	4
50 p.p.m. F	34	37.9	1	1
	35	41.5	2	4
	36	32.6	4	4

[a] Corrected to SpG of 1.04.

[b] Based on: 1 = questionable effect, 2 = slight deviation from normal, 3 = moderate effect, 4 = extensive changes.

Chemical evidence of the accumulation of fluorine in skeletal structures and elevated urinary levels are important in the evaluation of fluorine toxicosis. The urinary fluorine concentrations are presented in Table 3. Each value represents an average of two to three collections during each lactation. The data support our earlier observations; namely, that the normal excretion levels of the control cows were under 5 p.p.m. F, and there was a stepwise increase of urinary F with added increments of dietary fluorine. Further, the cows fed 50 p.p.m. F excreted urine which averaged greater than 30 p.p.m. F. At slaughter, representative samples of soft tissues and bones were obtained for chemical analysis. The materials were prepared for analysis and fluorine concentration determined by previously described methods (11). The data are presented in Table 4.

The changes in developing incisors caused by fluorine ingestion have received considerable attention (2, 6). However, in this experiment the cows

TABLE 4

Effect of level of dietary fluoride (NaF) upon bone and tissue fluorine concentration

Lot F added	Cow	Metacarpus	Metatarsus	Frontal	12th Rib	Heart	Liver	Kidney
(p.p.m.)		*(p.p.m. F dry fat free weight)*				*(p.p.m. F dry wt.)*		
I	25	345	282	403	283	1.7	2.7	2.8
	26	660	604	428	780	2.6	2.0	4.4
Control	27	545	545	573	567	2.3	....	3.3
	28	263	326	386	359	2.6	1.9	3.3
	Av.	453	439	448	497	2.3	2.2	3.5
II	29	2,630	2,510	2,780	2,450	4.8	2.4	10.4
	30	4,000	2,310	2,920	4,530	3.3	....	11.8
30 p.p.m. F	31	2,200	2,780	3,600	4,780	3.4	3.8	7.9
	32	2,120	2,780	3,290	2,440	3.8	2.3	12.3
	Av.	2,740	2,595	3,150	3,550	3.8	2.8	10.6
III	33	4,550	5,230	5,500	4,400	5.3	2.9	6.8
	34	3,460	3,570	5,480	4,910	....	3.3	11.1
50 p.p.m. F	35	4,460	4,780	5,370	6,200	3.6	1.5	8.3
	36	4,580	4,880	5,620	4,420	3.7	4.8	6.6
	Av.	4,200	4,620	5,490	4,980	4.2	3.1	8.2

possessed a full set of incisors when first subjected to elevated levels of dietary fluorine, and the incisors remained normal in appearance throughout the 3 yr.

The more critical physiological effects of fluorine toxicosis (9) were not observed in any of these animals. Even under the stress of initial heavy lactation there was no evidence of developing lameness, depression of appetite, or disturbance of milk production. Furthermore, the excessive post-partum weight losses observed in a previous study were not evident in these mature cows.

DISCUSSION

Because of the great similarity in experimental conditions, these data can be compared with the results of a previous experiment at this station (9, 10, 11), which utilized 2-yr.-old heifers which were under observation through five lactations. In the previous study (809A), inhibitory effects were noted on milk production and maintenance of adult body weight by appetite depression. In this study, neither milk production nor body weight was impaired by the elevated level of fluorine ingestion. This study (809B) indicated, as did the previous one, that urinary fluorine values for individuals vary greatly from one sampling to another, but if a sufficient number of determinations for each cow is grouped, the average value is, in general, related to intake. It is also evident that soft-tissue fluorine concentrations were extremely low and variable and were, therefore, of little or no value in diagnosis. The consistently higher values found in kidney tissue were presumably due to residual urine. The concentrations of fluorine in the urine and soft tissues in this mature cow study were very similar to those in the previous experiment.

This study further illustrates that the examination of incisors was of little or no diagnostic value if the animals were not exposed to fluorine during the period of incisor development.

It is of interest that the first appearance of a mild overcast exostosis of the long bones became apparent in this and the previous experiment at a dietary intake level of 30 p.p.m. F. In the present experiment, this intake level resulted in retention of from 2,500–3,000 p.p.m. F in the long bones, compared to an accumulation of 3,500–4,000 p.p.m. F in the previous experiment at the same level of intake.

The lower retention can be explained, in part, by the fact that in the mature cow study the animals were exposed for only three lactations, compared to five and one-half. It is also apparent that age has an influence on the rate of skeletal fluorine metabolism (3). To what extent this factor is involved is difficult to estimate, as a 2- and a 4-yr.-old skeleton are relatively the same size and may be considered mature. Any concentration of fluorine in the bones below 1,000 p.p.m. may be considered normal. It was concluded from the previous experiment that a range of from 4,500–5,500 p.p.m. F in the long bones was a concentration associated with a marginal zone of toxicosis. In general, animals with fluorine concentrations above this will begin to exhibit some systemic reaction to fluorine. This study supports those observations, since no such reaction was observed and the fluorine concentration of the metacarpal and metatarsal bones in the 50 p.p.m. F lot were just at the lower limit of the marginal zone. The evidence indicates no toxicosis due to fluorine.

Due to the difficulty in diagnosing moderate exostoses in the animal, no conclusion can be drawn as to the length of time needed to produce them in this or the previous study. In both cases, positive evaluation could not be made until the cows were slaughtered and the bones thoroughly cleaned of adhering tissue and examined.

It was previously demonstrated that when fluorine intake expressed on a body weight basis reached a level in excess of 1.7 mg. F/kg body weight there was a probability of the onset of a systemic reaction that was associated with a sudden loss of appetite. When animals received 50 p.p.m. F in the diet, this level was often exceeded as feed intake went up and body weight dropped at the beginning of heavy lactation. In this experiment, all of the cows in Lot III (50 p p.m. F) exceeded this level during their last lactation, by which time they should have been the most susceptible to the high F intake. Cow 35 reached a level of 1.95 mg. F/kg during its last two lactations. At no time was there any indication that these animals were adversely affected by these intake levels.

From these data it can be concluded that dairy cows first exposed to increased fluorine ingestion at maturity were able to tolerate the ingestion of as much as 50 p.p.m. F in the ration through three lactations without adverse physiologic effects. In contrast to this, it was previously shown that when cows were first exposed to 50 p.p.m. F at 2 yr. of age, temporary depression of appetite could be expected in certain cows in the third lactation.

It should be emphasized that the data presented were obtained under conditions of good nutrition and close confinement. Caution should be exercised in applying these results to other management conditions and practices.

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EFFECT OF FEEDING DIFFERENT RATIOS OF ROUGHAGE TO CONCENTRATE UPON MILK PRODUCTION AND DIGESTIBILITY OF THE RATION

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SUMMARY

A randomized block design was used to study the productive and digestive efficiency of cows consuming rations containing 80, 60, and 40% dry matter from roughage and the balance from concentrates. Twelve animals were used and the experimental period was 112 days in length. Digestibility trials, involving total fecal collections, were conducted at about the 10th and 20th wk. of lactation. In addition, eight dry cows were used for obtaining digestibility values on the roughage portion of the ration.

As the proportion of concentrate in the ration became larger, the apparent digestibility coefficients of the mixed ration increased for dry matter, crude protein, ether extract, N-free extract, and total carbohydrates (crude fiber plus N-free extract). Total digestible nutrient and dry matter intakes also increased. The apparent digestibility of crude fiber decreased as the proportion of concentrate in the ration increased. There was no significant difference in the average amounts of milk produced by the animals consuming the different rations. Efficiency of milk production, expressed as pounds of total digestible nutrients per pound of 4% fat-corrected milk produced, decreased as the proportion of concentrate in the ration increased.

The data support other reports which suggest that present U. S. feeding standards for dairy cattle are not as accurate at high or low levels of production as at medium levels, and that they are more accurate with usual rations than with very high proportions of either concentrates or roughage.

Dairy farmers and research workers would like to be able to predict the optimum levels of concentrate feeding at various feed and milk prices. Before accurate predictions can be made, the physiological response to changing concentrate levels must be well defined. Data from balance studies carried out in conjunction with feeding trials can contribute to the solution of the problem, but relatively few digestion-lactation studies have been carried out. Instead, digestibility values are often obtained from studies with sheep or steers being fed at maintenance levels. Average values from standard tables are also used, as are values calculated from regression equations. Eventually, the determinations must be made with high-producing dairy cows.

In a recent review on input-output relationships, Jawetz (11) concluded that "there is sufficient evidence for a revision of the conventional recommendations of production requirements per unit of milk" and that "these requirements are proportionally more at high levels of yield than at low levels." This conclusion is supported by evaluation of the data of Bloom *et al.* (4) by Ibbotson (10).

The literature concerned with feed input—milk output relationships has also been reviewed by Blaxter (2), Reid (19), and Burt (5). Blaxter (2) speculated that part of the diminishing returns from increasing feed inputs may be due to a decline in digestibility. At a much earlier date, Schneider and Ellenberger

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(20) had expressed the opinion that digestion trials should be conducted with all feeding trials, so that digestible nutrient intakes can be accurately computed.

The few experiments with dairy cows which have been directly concerned with the digestibility of rations containing varying ratios of roughage to concentrate have revealed the expected increase in the digestibility of dry matter, ether extract, nitrogen-free extract, and an increase in TDN content of rations containing the higher levels of concentrates (3, 9, 12, 18, 22). The digestibility of crude fiber has been less clearly affected, but has shown a tendency to decrease with increasing levels of concentrates. Similar results with beef cattle and sheep have been reported (6, 15, 17).

Thus, the literature indicates that in experiments when lactating dairy cows are fed various roughage to concentrate ratios, several trends may be expected. Milk production and digestibility coefficients (except for crude fiber) should increase as the proportion of concentrate in the ration increases. Conversely, milk unit returns per feed input unit should diminish as total feed inputs and milk production increase. As plane of nutrition increases, digestibility coefficients frequently decrease. Similarly, the percentage of fat in the milk frequently decreases as the proportion of grain in the ration increases, especially when the roughage intake becomes too low. Finally, expected milk yields based on feeding standards and average digestibility values do not coincide with actual production values at the highest levels of intake.

The need of conducting digestibility studies with high-producing dairy cows is apparent. Since wide variations in plane of nutrition are achieved in practice by increasing the amount of concentrate in the ration, this method was followed in studying the efficiency of feed utilization. The experiment was designed to gain information on digestibility and milk production by dairy cows consuming rations differing widely in roughage to concentrate ratios with ad libitum feeding.

EXPERIMENTAL PROCEDURE

A randomized block design was used to study the productive efficiency of purebred Holstein cows fed rations containing 80, 60, and 40% dry matter from roughage. Twelve cows were stratified into four subgroups on the basis of previous milk production, fat test, and age, and randomly assigned to treatment groups. The experiment consisted of a 14-day adjustment period during the 7th and 8th wk. of lactation, followed by a 112-day experimental period during the winter of 1956–57. The experimental design is shown in Table 1.

TABLE 1

Design used in the lactation study

Stratification	Per cent roughage		
	80	60	40
	(cow numbers)		
1	174	150	251
2	180	166	194
3	164	153	193
4	196	254	279

Second cutting, legume-grass, mixed hay, and well-matured, well-eared corn silage were fed at a ratio of 1 lb. of hay to 3 lb. of silage. The concentrate mixture consisted of the following percentages: ground yellow corn, 21; ground oats, 20; wheat bran, 10; corn distillers dried grains, 40; cane molasses, 7; dicalcium phosphate, 1, and salt, 1.

All feeding was based on ad libitum consumption at each roughage to concentrate ratio. The cows were fed so that they left 1–2 lb. of hay or silage each day, but when the refused feed exceeded that amount adjustments were made to keep the desired ratios of hay to silage and of roughage to concentrate. If the cows left less than 1 or 2 lb. of roughage, larger amounts of feed were offered.

The animals were housed in a conventional stanchion barn except during digestion trials. They were allowed to exercise 2–3 hr. each day in a paved yard. All feeds were fed twice daily and records of refused hay, silage, and grain were kept, as were the records of milk produced. The cows were milked twice daily and a composite sample from four consecutive milkings each week was analyzed for butterfat content by the Babcock method. Feed samples were taken daily during digestibility studies and analyzed by A.O.A.C. methods (1). These analyses of the feeds were applied also to the lactation experiment.

Digestion trials were run with each of the lactating cows at approximately the 10th and 20th wk. of lactation. Eight dry or nearly dry cows were used to determine the digestibility of the roughage component of the ration. The animals were moved from the main barn to special stalls a week before fecal collections were to be made. Since the same feeding system was followed during the digestion trials and during the rest of the lactation study, there were small day-to-day variations in actual feed consumed. Therefore, average daily intakes during digestibility determinations were calculated on the basis of the feed intakes during the collection and the five days prior to collection.

RESULTS AND DISCUSSION

Lactation trial. The average chemical composition of the feeds is presented in Table 2.

The procedure of full-feeding, as determined by the occurrence of refused feed, proved quite satisfactory. Although the amounts of orts were not constant for all animals (Table 3), the average amounts approximated the planned 2.0 lb. of dry matter per day. The percentage of hay equivalent in the ration and the ratio of hay to silage were approximately as planned. If the corn grain in the

TABLE 2

Average chemical composition of feeds

Feed	Dry matter	Crude protein	Ether extract	Crude fiber	N-free extract	Ash	Air-dry matter
	(%)						
Hay[a]	88.0	15.1	1.9	27.6	37.6	5.8	
Silage	93.7	9.4	2.4	28.3	49.0	4.6	23.0
Concentrate mixture	88.4	16.8	4.4	9.5	53.6	4.2	

[a] The hay was graded U. S. No. 1 with 40% color. Botanically, the sample contained 37.7% alfalfa, 49.2% clover, 13.1% timothy, and 8.5% foreign matter.

TABLE 3

Average daily feed consumed and refused and per cent roughage consumed

Per cent roughage planned	Hay		Silage		Concentrate		Per cent roughage consumed (dry basis)	
	Consumed	Orts[a]	Consumed	Orts	Consumed	Orts	[b]	[c]
	(lb.)							
80	17.3	1.6	54.8	2.0	8.7	0.0	77.8	68.0
60	14.0	1.5	42.0	3.2	17.9	0.1	57.4	50.3
40	10.1	0.6	30.2	1.4	26.5	0.4	39.4	34.8

[a] Refused feed.
[b] Corn silage considered entirely roughage.
[c] Corrected for the grain in the corn silage.

silage is estimated (16) and 75% of the dry matter of the ears is considered as part of the concentrate, the rations studied contained 68.0, 50.3, and 34.8% roughage instead of 80, 60, and 40%.

The average daily production of milk, fat, and FCM, and the fat percentage, TDN intake, body weight, and FCM per pound TDN are presented in Table 4. The average fat test and average daily production of 4% fat-corrected milk for the cows consuming the 80, 60, and 40% roughage rations was 3.69, 3.44, and 3.58% and 48.1, 49.3, and 52.6 lb., respectively. An analysis of variance of the weekly total FCM production showed that there was no significant difference in the average amounts of milk produced by the animals consuming the different rations (Table 5). However, the significant interaction between treatments and weeks indicates that the effectiveness (in stimulating milk production) of large proportions of grain in the ration diminished as the lactation period progressed. There was no difference in the FCM production during the pre-experimental period between the groups that were assigned to the various experimental rations. The mean differences between stratification groups during the experimental period were also not significant, as indicated by using the T × S mean square as the appropriate error term.

Digestion trials. The results of the digestion trials are presented in Tables 6 and 7. Dry matter intakes were greater when there was a high proportion of concentrates in the ration. The coefficients of apparent digestibility for dry matter, crude protein, ether extract, N-free extract, and carbohydrate (crude fiber plus N-free extract), as well as TDN values, increased as the proportion of

TABLE 4

Average daily production of milk, fat, and FCM, and the fat percentage, TDN intake, body weights, and FCM per pound TDN [a]

Per cent roughage	Milk	Fat	Fat	FCM	TDN intake	Average weight	FCM per lb. TDN
	(lb.)	(%)	(lb.)				
80	50.4	3.69	1.86	48.1	22.9	1,363	2.10
60	53.9	3.44	1.85	49.3	25.4	1,356	1.94
40	56.2	3.58	2.01	52.6	27.3	1,352	1.93

[a] During the 112-day experimental period.

TABLE 5

Analysis of variance and coefficients of variation for 4% fat-corrected milk production during the pre-experimental and experimental periods

Source of variance	Degrees of freedom	Mean squares for the pre-experimental period (3rd through 6th wk.)	Degrees of freedom	Mean squares for the experimental period (8th through 24th wk.)
Treatment groups (T)	2	1,540.5	2	17,862.9
Stratification groups (S)	3	18,328.9	3	11,423.1
Weeks (W)	3	5,307.6	15	14,330.8**
T × W	6	884.6	30	5,210.8**
S × W	9	670.9	45	552.4
T × S plus cows	6	5,592.7**	6	15,996.9**
Residual	18	1,048.3	90	652.9
Coefficient of variation (residual m.s.)		7.6		7.3
(T × S plus cows m.s.)		17.6		36.1

TABLE 6

Average daily feed consumed and feces voided in the digestion trials

Per cent of roughage		Feed consumed				
Planned	Actual	Hay	Silage	Concentrate	Total	Feces
		(lb. dry basis)				
100	100	13.7	10.0	0.0	23.7	8.2
80	78.6	15.6	11.6	7.4	34.6	11.8
60	56.8	12.4	9.2	16.2	37.8	12.2
40	39.9	8.9	6.5	23.0	38.4	12.1

TABLE 7

Average digestion coefficients, total digestible nutrient values, and coefficients of variation

Per cent	Dry matter	Crude protein	Ether extract	Crude fiber	N-free extract	Carbohydrates[d]	TDN
100	65.2[a]	69.8[a]	60.9[a]	59.6[a]	70.5[a]	66.2[a]	64.6[a]
80	66.1[a,b]	69.7[a]	69.1[b]	57.0[a]	72.0[a]	66.8[a]	66.1[a]
60	67.6[b,c]	71.3[a,b]	74.1[c]	53.8[b]	74.2[b]	68.0[a,c]	68.5[b]
40	68.6[c]	72.4[b]	77.0[c]	51.9[b]	74.8[b]	69.0[c]	70.6[c]
Coefficient of variation	2.30	2.61	5.31	4.98	2.63	2.55	2.40

[a,b,c] Values without a common subscript and in the same column are significantly different according to Duncan's multiple range test (8).

[d] Carbohydrates equal crude fiber plus N-free extract.

concentrate in the ration increased. The coefficients of apparent digestibility for crude fiber decreased as the proportion of concentrate in the ration became greater. There was a high positive correlation between the average percentages of crude protein, ether extract, crude fiber, and N-free extract in the rations and their corresponding average digestion coefficients ($r = +0.96$, $+0.94$, $+0.99$, and $+0.98$, respectively). The average digestibility coefficients and coefficients of variation are presented in Table 7. Statistically significant differences between treatments were observed within each of the classes of nutrients studied.

The coefficients for dry matter digestibility and the TDN values reflect

changes in the digestibility of their component nutrients. No zone of "unfavorable nutrient combination" was observed, as suggested by Bloom *et al.* (3).

The differences in the digestibility of crude protein are probably more apparent than real and, since the lowest protein intakes were well above recommended levels, any variation in the protein intakes is not considered important (5).

The changes in the digestibility of ether extract, apparent or real, are quantitatively of little importance. Similarly, the importance of the decreases in the digestibility of crude fiber as the proportion of the concentrates in the ration increases is minimized because of the concurrent increase of the total carbohydrate (crude fiber plus N-free extract) coefficients.

The crude fiber digestion coefficients were much higher than those reported by Bloom *et al.* (3).

The data in Table 8 show that the TDN values of the concentrate, calculated by difference, are the same at the various levels of roughage intake. Statistical analyses of the variation due to treatments show that, with the exception of digestibility coefficients for the ether extract, the majority ($P > 90\%$) of the variation is linear in nature. These observations suggest that within the range studied (100 to 40% roughage or at TDN intakes from 2.5 to 3.0 times that necessary for body maintenance) there is no detectable change in the digestion of the nutrients which can not be explained by the theory of additive digestibility of roughage and concentrates. However, while digestion is qualitatively a very important process in the utilization of feed (energy), it is only the initial step. From the productive aspect, the utilization of the digested nutrients is perhaps even more important.

The total digestible nutrient intakes during the lactation study have been calculated from the TDN values determined in the digestion studies. In many feeding trials, average values for TDN are used. Thus, for comparative purposes total TDN intakes were estimated, using Morrison's tables (14). TDN values for alfalfa hay, leafy (25–28% fiber); clover hay, red, all analyses; and timothy hay, all analyses, were averaged according to their respective proportions in the hay mixture (Table 2). Corn dent, well-matured, well-eared, was considered representative of the silage. The resulting TDN value for the roughage was 64.1 on a dry basis. The value of the concentrate was determined similarly and it was estimated that it contained 85.1% TDN on a dry basis. The formulas of Schneider

TABLE 8

Total digestible nutrient value of the concentrate as determined by difference

Trial[a]	Per cent concentrate in the ration		
	20–25	45–50	60–65
I	82	78	77
IIa	65	73	74
IIb	78	75	75
Average $\pm S_{\bar{x}}$	75 ± 5.3	75 ± 1.3	75 ± 0.7

[a] Trial I refers to Putnam and Loosli (18). Trials IIa and IIb are the 10th- and 20th-wk. collections reported in the text.

et al. (21) were also used to estimate TDN. The values obtained for the roughage and concentrate were 56.2 and 80.8, respectively.

The experimentally determined and estimated TDN intakes were then compared with predicted TDN intakes, based on the NRC requirements (13) for cows of the same body weight and producing the same amounts of milk (Figure 1). It is readily observable from the experimental values that the 80% roughage group consumed about 6% less, and that the 40% roughage group consumed about 6% more, TDN than calculated requirements on the basis of their respective production levels. Similar trends were observed by Ibbotson (10), whose report suggests that our present feeding standards are too low for high production and too high for low production. While the same trends are described when estimated TDN values are used, the absolute values were quite different. This supports the contention of Schneider and Ellenberger (20) that "average figures are to be questioned when applied to individual cases in any kind of exact experimental work."

These results suggest that there was a decreased efficiency in the utilization of TDN for milk production as the TDN (concentrate) consumed increased. Calculations indicated that the pounds of TDN consumed per pound of 4% fat-corrected milk increased as the amount of TDN consumed increased, and the

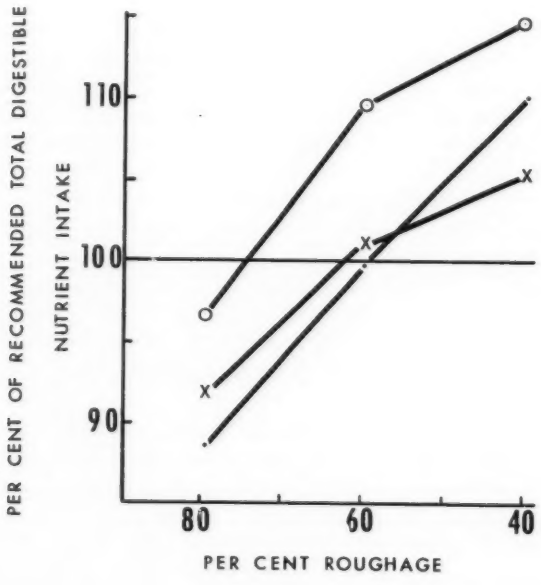

FIG. 1. Estimated [(⊙—⊙, Morrison's tables, •--•, Schneider's formulas) and observed (×) total digestible intakes by animals consuming various percentages of roughage in their rations (expressed as per cent of the recommended intakes)].

difference was significant ($P < 10$). Average body weight changes from the first half to the last half of the experiment were not significantly different for the animals on the various rations. Whether changes in efficiency were due to changes in gaseous losses or some other factor can not be determined from the data. The recent report by Elliot and Loosli (7) suggests that it is not the effect of a change in ratio per se and, thus, one would suspect plane of nutrition as being involved in the decreased efficiency. Whatever the mechanism involved, the decreased efficiency observed is in agreement with the more extensive works cited in the literature and does not seem to be due to a decreased digestibility.

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INFLUENCE OF ARTIFICIAL BREEDING ON PRODUCTION IN MICHIGAN DAIRY HERDS [1]

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SUMMARY

Daughters of bulls in artificial breeding units were compared with non-A.B. heifers in the same herds at the same time, to assess the genetic progress in production achieved by artificial breeding in Michigan herds on D.H.I.A.—I.B.M. test. Holstein A.B. heifers produced 124 lb. more milk and 3.7 lb. more fat in their first lactation than did their non-A.B. contemporaries. Guernsey non-A.B. heifers exceeded A.B. heifers by 6 lb. of milk, but produced 3.9 lb. less fat. Jersey A.B. heifers produced 16 lb. more milk, but had 2.7 lb. less fat.

The differences, A.B. heifers minus non-A.B. heifers, tended to increase slightly but not significantly as the average fat production of other contemporary cows increased, except for Jerseys, where they tended to decrease slightly.

No interaction between A.B. sires and herds existed when each A.B. daughter's record was expressed as a deviation from the average of all non-A.B. cows in the same herd.

The influence of artificial breeding on genetic improvement in dairy herds needs constant review to guide future plans of this increasingly popular industry. Over 277,000 cows were bred artificially in Michigan in 1957, nearly one-third of the cows and heifers kept for milk. Production of butterfat in tested herds has been increasing about 3 lb. per year. This report is an appraisal of the influence of artificial breeding on production in Michigan dairy herds on D.H.I.A.—I.B.M. test.

Investigation of the influence of artificial breeding is restricted to those herds which have records of production. Only a small percentage of cows are tested and a small proportion of these are artificially bred. How the results from this special group of tested herds relate to the influence of artificial breeding on untested herds, which probably produce less than the tested herds, is uncertain.

Appraising the effect of artificial breeding can be done initially by comparing daughters produced by artificial breeding with daughters of bulls used in natural service, in herds which have both types. The results of artificial breeding in Michigan D.H.I.A.—I.B.M. herds were measured by mean weighted differences between the average production of heifers obtained by artificial breeding and the production of other heifers in the same herd. The mean weighted difference (MWD) is the average performance of the artificial daughters minus the average performance of the natural daughters, weighted by the reciprocal of the variance of this difference. Robertson and Rendel (6), comparing 1,423 daughters from artificial breeding with their herd-mates from bulls used naturally, concluded that the milk production of A.B. daughters differed little from non-A.B. daughters in Great Britain. In New Zealand (5) 1,010 A.B. two-year-old heifers produced 10 lb. of fat more than their naturally bred herd-mates.

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The related questions of whether the differences between A.B. and natural cows are affected by the level of production in the herd, and whether the comparisons of A.B. daughters with their herd-mates for a particular bull vary significantly from herd to herd, were also examined. Robertson and Rendel (6) found no general trend in the comparisons as the level of production of the herd changed. Mason and Robertson (4) found no evidence of herd-sire interaction for annual milk production, either within or between levels or management.

SOURCE OF DATA

The records used were a part of the D.H.I.A.—I.B.M. records collected in Michigan from October, 1954, to June, 1956. Only completed records which were at least 180 days long were included. All records over 305 days were terminated at 305 days. Those herds in which artificial and natural daughters appeared were used.

DIFFERENCES BETWEEN A.B. AND NON-A.B. HEIFERS

The herds involved in the MWD were those herds in which both artificial and natural daughters had first-lactation records. MWD's were computed for actual milk, actual fat, mature equivalent milk, and mature equivalent fat. Where a distinction was made between grade and registered herds, a herd with any grade animals was classified as a grade herd, and a registered herd contained only registered animals. Where a herd was composed of several breeds, it was broken into herd-breed groups and each classified as a separate herd. The procedure followed in making the calculations was that set forth by Robertson and Rendel (6).

Table 1 shows for registered and grade breed groups the mean weighted

TABLE 1

Differences in production between A.B. daughters and their non-A.B. contemporaries in the same herd

Breed	No. of herds	No. of artificial records	No. of natural records	Mean weighted differences (in lb.)			
				Actual		M.E.	
				Milk	Fat	Milk	Fat
All Holstein	176	558	864	96	2.9	124	3.7
Grade Holstein	142	447	678	25	0.6	60	1.7
Registered Holstein	34	111	186	366	11.8	370	11.4
All Guernsey	33	116	197	−10	2.8	−6	3.9
Grade Guernsey	25	96	114	−21	1.6	−27	2.1
Registered Guernsey	8	20	83	29	6.9	71	10.5
All Jersey	17	54	73	49	−0.7	16	−2.7
Grade Jersey	12	44	38	113	3.3	43	−0.6
Registered Jersey	5	10	35	−73	−8.3	−34	−6.6

differences in production between A.B. daughters and their non-A.B. contemporaries in the same herd. Holstein A.B. heifers produced 24 lb. more milk and 3.7 lb. more fat than their non-A.B. contemporaries. Guernsey non-A.B. heifers exceeded A.B. heifers by 6 lb. of milk, but produced 3.9 lb. less fat. However, Jersey A.B. heifers produced 16 lb. more milk than the non-A.B. heifers, but had 2.7 lb. less fat. These differences are similar in size to those observed by Robertson and Rendel (6).

Level of production. An analysis of variance was used to determine whether the MWD's were significantly different among various levels of production of herds. Herds were assigned to levels of production on the average M.E. fat of the cows in the herd at the same time, but not used in the previous comparison. The herds were divided into ranges of 50 lb. of M.E. fat, i.e., 250-299, 300-349, etc. Comparisons were then made of the differences between A.B. daughters and non-A.B. daughters in actual milk, actual fat, M.E. milk, and M.E. fat.

The results in Table 2 show no significant trend for A.B. daughters to produce

TABLE 2

Differences between A.B. daughters and their natural contemporaries high- and low-producing herds

M. E. butterfat range	No. of records			Mean weighted differences			
				Actual		M.E.	
	Herds	Natural	Artificial	Milk	Fat	Milk	Fat
Guernsey							
250–299	1	1	1	−4,366	−44	−4,366	−22
300–349	12	51	48	−169	−6	−195	−7
350–399	9	43	25	−322	−12	−332	−10
400–449	6	51	19	775	38	773	37
450–499	5	51	23	73	3	152	8
Holstein							
250–299	1	4	1	−2,465	−111	−2,634	−124
300–349	11	28	30	501	28	679	37
350–399	38	207	116	−38	−1	−30	−1
400–499	45	236	165	93	3	144	4
450–499	47	245	136	−164	0	−244	−1
500–549	22	84	76	658	10	718	8
550–599	8	38	28	124	−5	286	−2
600–649	2	19	2	453	34	1,071	63
Jersey							
200–249	1	1	1	1,744	96	2,144	120
300–349	3	10	12	169	23	148	25
350–399	7	31	17	−321	−26	−451	−35
400–449	4	18	20	430	9	403	5
450–499	1	6	1	−74	−33	28	−34
500–549	1	7	3	−376	1	−249	14

less or more than contemporary non-A.B. daughters in the same herd as other cows in the herd vary in average production. Differences in actual milk for the Guernsey breed are significantly different at the 5% level. In all other cases, no significant difference appeared between the MWD's at different levels of production. In the Holsteins, the analysis of variance gave a value of F of less than one in all cases.

A regression analysis of these same data with the independent variable being the average M.E. of the older herd-mates and the dependent variable being the MWD for M.E. butterfat gave the following regression coefficients: Guernsey 16, Holstein 1, and Jersey 16. Standard errors of estimate were Guernsey 78, Holstein 79, and Jersey 54. The regression coefficients were not significantly different from zero at the 5% level. Confidence intervals at the 5% level for the regression

coefficients are: Guernsey − 4 to 37, Holstein − 6 to 8, and Jersey − 36 to 5. These results were tested for linearity and no evidence was found that the relationship was not linear.

HERD-SIRE INTERACTION

The herd-sire interaction and other components of variance, sires, herds, and residual were obtained by equating mean squares to expectations in an analysis of variance of records from herds which had both artificial and natural daughters. Each artificial record was expressed as a deviation from the average of all natural cows in the same herd. Repeated records of both artificial and natural daughters were eliminated in a random manner. Computations were completed on M.E. milk and M. E. fat.

No herd-sire interaction was found in this study. The results are shown in Tables 3, 4, and 5. In all cases, the component for herd-sire interaction was

TABLE 3
Analysis of variance and components for Guernseys

Source of variance	D.F.	M.S.		Variance components			Per cent of total	
		Milk*	Fat		Milk*	Fat	Milk	Fat
Total	257							
Sire	73	3,414**	6,391**	S	330	340	14	6
Herd	58	5,054**	10,797**	H	794	1,698	33	30
Registered vs. grade	1	12,024**	31,177**					
Between herds within class	57	4,931**	10,439**					
Herd × sire	67	−117	388	HS	10	−418	0	0
Sire × registered: grade	4	826	7,408					
Remainder	63	−177	−57					
Error	59	1,278	3,620	E	1,278	3,620	53	64

* Mean squares and components of milk coded in thousands.
** Significant at the 1% level (approximate test).

TABLE 4
Analysis of variance and components for Holsteins

Source of variance	D.F.	M.S.		Variance components			Per cent of total	
		Milk*	Fat		Milk*	Fat	Milk	Fat
Total	1,496							
Sire	198	6,950**	7,034**	S	244	46	5	1
Herd	281	9,568**	11,267**	H	1,058	1,161	21	17
Registered vs. grade	1	6,028	13,422					
Between herds within class	280	9,581**	11,259**					
Herd × sire	771	3,200	4,744	HS	−467	−415	0	0
Sire × registered: grade	60	6,918**	−21,096					
Remainder	711	2,887	6,784**					
Error	246	4,233	5,548	E	4,233	5,548	84	82

* Mean squares and components of milk coded in thousands.
** Significant at the 1% level (approximate test).

either negative or nearly zero. Negative interaction components are obtained when the herd-sire groups are found to be more alike than expected. These results compare favorably with those of Mason and Robertson (4) and Legates *et al.* (3). These results show that sires will be ranked about the same, regardless of the groups of cows with which they are mated.

TABLE 5
Analysis of variance and components for Jerseys

Source of variance	D.F.	M.S.			Variance components		Per cent of total	
		Milk*	Fat		Milk*	Fat	Milk	Fat
Total	92							
Sire	24	2,801	7,478	S	454	1,234	13	12
Herd	20	3,803	10,844	H	580	1,689	17	16
Registered vs. grade	1	1,048	143					
Between herds within class	19	3,948	11,407					
Herd × sire	17	−1,260	−3,707	HS	−1,280	−4,302	0	0
Sire × registered: grade	4	1,611	7,586					
Remainder	13	−2,141	−7,182					
Error	31	2,369	7,490	E	2,369	7,490	70	72

* Mean squares and components of milk coded in thousands.

The variance due to herds (H) ranged between 16 and 33%, variance due to sires (S) between 1 and 14%, and error (E) variance between 53 and 84% with no herd-sire interaction (HS). In all breeds, H falls below the upper bound of those figures obtained by Gaunt and Legates (2), and Legates *et al.* (3). However, H for Holstein fat and for Jersey milk and fat falls below previously found figures. The results for S indicate that the Holstein sires used were of almost equal genetic value, and that the Jersey sires used varied considerably in their genetic value. The variation between daughters of the same sire in the same herd (E), due to such things as variation in dams and environmental causes, accounted for the major portion of the total variance. Except for the Guernsey breed, E is much larger than reported before. The E for the Guernsey breed is smaller than previously observed.

DISCUSSION

The herds involved in this study are above-average herds of Michigan, which can be seen by comparing the production level of these herds shown in Table 6 with the production of the average cow in Michigan, 6,500 lb. of milk. The average production of these herds in all instances is well above this figure.

TABLE 6
Average 305-2×-M.E. production for all breeds

Breed	All D.H.I.A.—I.B.M. cows			Cows studied		
	Number	Milk	Fat	Number	Milk	Fat
Holstein	13,899	11,835	435	5,360	12,170	438
Grade	7,401	11,701	420	2,921	11,841	427
Registered	6,498	12,680	453	2,439	12,565	451
Guernsey	3,037	8,240	391	1,001	8,136	389
Grade	1,491	8,137	379	490	7,844	370
Registered	1,546	8,339	402	511	8,400	407
Jersey	1,677	7,559	393	468	7,594	400
Grade	792	7,483	384	208	7,632	401
Registered	885	7,628	401	260	7,564	400

As shown previously, the over-all MWD between A.B. daughters and non-A.B. daughters is positive. However, nearly 50% of the herds in this study have negative MWD's. The small differences in production between A.B. daughters and non-A.B. daughters within the same herd seem to indicate that the sires used by the breeding studs have been little, if any, genetically superior to the sires previously used naturally. Before using artificial breeding, 83% of the herds included in this study were using a registered sire or a bull by a registered sire. Forty-four per cent of those herds that today are using artificial and natural sires concurrently are using naturally a registered sire or a bull by a registered sire. The source of the sires used naturally could have been the same as the source of sires used by the breeding studs.

The interpretation of the small differences between A.B. and non-A.B. heifers would differ considerably from the conclusions suggested if these groups were produced by differential matings or were managed differently within the herds. There were no differences between the production of the dams of A.B. and non-A.B. heifers (1). The age and body weight at first calving did not differ significantly between the two groups. It is doubtful that the feeding of the two groups after calving would differ, although this was not examined.

Although the source of breeding bulls for these herds on D.H.I.A.—I.B.M. may have been similar, in part, to the source of bulls used in artificial breeding units, the bulls used artificially have usually come from the more popularly known herds engaged in selling breeding stock. It appears from these results that if the sources of bulls for natural service and for artificial service really are different, they differ little in genetic merit for production. What differences in production do exist fall mostly to differences in management and general environment.

The regression of the MWD's on herd averages indicates an increase in MWD's for an increase in herd average for the Holstein and Guernsey breeds, but a decrease for Jerseys. Before this study, it had been thought that differences in favor of A.B. daughters would increase to a certain plane of production and then level off or decrease, giving a curvilinear relationship between herd averages and MWD's. At a low level of production the benefits of an artificial sire could be cancelled out by the environment, giving a greater chance for a smaller or negative MWD. At high levels there might be less opportunity for A.B. bulls to be genetically superior to the herd bulls. The study, however, showed a linear relationship with no tendency to become curvilinear in the way suggested. Except for the Jerseys, the MWD's tend to get slightly larger as the herd level increases, but not significantly so. This may reflect only the increase in size of differences between progeny groups with increased level of production in herds, as observed by Mason and Robertson (4).

The study supported, by showing no herd-sire interaction, the fact that artificial sires will rank in about the same order in the herds in which their daughters appear. This does not mean, however, that to express the daughter's production as a deviation from the herd average in which it appears may not be useful when comparisons are made. All bulls will not have daughters in all herds, and using

the daughter's production as a deviation from the herd average may aid in eliminating a portion of the variance due to herd environment and to yearly environmental conditions.

These results may not apply directly to herds at different levels of production from those herds in this study. The variation of environmental conditions overshadows the importance of the sires, herds, and herd-sire interaction. This may explain why there is not a larger advantage for A.B. daughters at the lower levels of production. Along with lower levels of production, in many instances, goes a poor environment. Assuming that the herds at the lower level of production are not at the same level genetically as the breeding herds, we could expect the A.B. sires to help at a lower level of production to a greater extent if the environment were not such to cover up the effects.

More effective programs of selecting sires than have been used in the past appear to be needed if artificial breeding is to offer the type of herd on D.H.I.A. test an opportunity to improve genetically more rapidly than they can by selecting their own herd sires.

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ESTRUS, ESTROUS CYCLES, OVULATION TIME, TIME OF SERVICE, AND FERTILITY OF DAIRY CATTLE IN LOUISIANA [1]

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SUMMARY

The mean duration of estrus for 1,460 estrous periods for 270 cows and heifers was 11.9 hr., with heifers having significantly longer estrous periods than parous cows. Nonstanding estrus was observed in 10.1% of the estrous periods. Estrus after conception was exhibited by 14.4% of the animals and involved 3.4% of the estrous periods. Ovulation occurred 12.4 hr. after the end of estrus. Optimum time for insemination was 7–12 hr. after the onset of estrus. Anestrus occurred one or more times in 33.4% of the animals, representing 10.6% of the expected estrous periods. It was calculated that anestrus would have increased 19.9% with only 0600 and 1800 hr. observations; whereas, an additional check at 1,200 hr. increased the incidence only 10.0% above that which occurred during 6-hr. observational periods. Monthly PBI levels were inversely related to environmental temperature and humidity.

Basic information concerning the duration of estrus, estrous cycle length, time of ovulation, and optimum time of service is needed, to obtain maximum fertility both in natural and in artificial breeding of cows. Considerable information relative to such needs has been accumulated from the temperate regions of the world (2, 3, 4, 5, 7, 17, 20, 27), but information from the subtropics and tropics (1, 10, 13) is scarce and based on small numbers of animals and experiments of short duration. These limited data from the tropics and subtropics suggest that estrous periods are about 5 hr. shorter in such areas than in the temperate areas of the world. It was, therefore, reasoned that the high incidence of anestrus in Louisiana (6, 18) possibly was due to short estrous periods which were not being observed. Some investigators have theorized that climate has a depressing effect on the expression and duration of estrus, since high environmental temperatures and humidities have been found to be inversely correlated with fertility both in the bull (12) and in the cow (21). It has also been postulated that the cows with short estrous periods perhaps were being bred too late for optimum rate of conception.

Because of this lack of data, the investigations reported herein were undertaken with the following objectives: (a) to characterize the estrous periods and cycles of dairy cattle under Louisiana conditions, (b) to interrelate estrus, estrous cycles, ovulation time, thyroidal activity, and fertility in such animals, and (c) to ascertain the nature of anestrus in dairy cattle in Louisiana.

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EXPERIMENTAL MATERIALS AND METHODS

A total of 270 cows and heifers, including 151 Holsteins, 95 Jerseys, and 24 Red Sindhi–Holstein crossbreds (F_1 and $\frac{3}{4}$ Holstein–$\frac{1}{4}$ Sindhi) in the Louisiana State University dairy herd were observed during the period from January 1, 1957, through May 31, 1958. Feeding and management practices followed during this period approximated those followed by most good commercial dairymen in Louisiana. However, the forage feeding program was not considered to be optimum in terms of quality and quantity during much of this time for maximum milk production and for the maintenance of body weights in this herd. Many of the animals lost weight during the fall and winter months. The temporary and permanent pastures and feeding lots allowed free movement of the animals, but were sufficiently small to permit efficient observations for estrus. No animals were housed at any time except for a brief period before parturition and for about 2 hr. during the milking operation.

Measurement of estrus. Observations for estrus were made at 0600, 1200, 1800, and 2400 hr., except during four seasonal ten-day periods, when observations were made at 2-hr. intervals. Estrus expectancy lists were used as an aid in the detection of estrus. The intensity of estrus was scored each time it was observed within each estrous period. A score of 1 was given those periods during which symptoms such as a thin, glary vaginal discharge, nervousness, and an unusual interest in herd-mates were manifested. A score of 2 was given for more intense periods with an increased amount of vaginal discharge, considerable excitement, and mounting of other females. A score of 3 was given when the animal stood when mounted, with or without the symptoms given for Scores 1 and 2. Standing when mounted was not considered an essential criterion of estrus. Animals showing nonstanding estrus were palpated rectally for ovarian activity, to verify such estrous periods. Estrous periods following hormonal treatment, abortion, and severe illness were removed from the data. Hormonal treatment was used sparingly and only for cows which had not shown estrus by 90 days postpartum or had missed at least two estrous periods and had nonfunctioning ovaries. The hormones used were diethylstilbestrol, estradiol, and progesterone. Some of the persistent corpora lutea were expressed manually by the herd veterinarian. The data on sterile heifers were removed, as recommended by Branton *et al.* (6).

Anestrus. Anestrus was determined by the negative approach with both physiological and psychological anestrus grouped together and considered as clinical anestrus. Estrus cycles which were abnormally long and included multiples of normal estrous cycles were considered to include anestrous periods.

Breeding practices. All animals were bred artificially. Cows were bred during the estrous period nearest 60 days postpartum. Heifers were bred according to age, with some attention given to size. A breeding recess was observed during the months of July, August, and September, 1957. Pregnancy diagnoses were made by rectal palpation from 45 to 75 days following an apparent conception.

Ovulation time. The 51 animals on which ovulation time was determined were

selected at random from those cows and heifers exhibiting estrus during certain week ends. Palpation was begun at time of insemination and continued thereafter at 6-hr. intervals until ovulation had occurred.

Thyroidal activity. Thyroidal activity was measured in terms of blood serum protein-bound iodine (PBI) levels. Jugular blood samples were taken from 26 cows at monthly intervals (15) and analyzed for PBI by the Hycel[3] method as described by Moran (19).

All statistical analyses of the data were performed according to the methods of Snedecor (26), except for group means, which were tested by Kramer's (16) modification of Duncan's multiple range test.

RESULTS AND DISCUSSION

Characteristics of estrus. Since the average duration of estrus of 11.8 hr., obtained during the 2-hr. observational periods, was essentially the same as that obtained during the 6-hr. observational periods, these data were combined and summarized as shown in Table 1. It appeared that the additional labor to carry out the 2-hr. observations was not justified in terms of economy or accuracy of estimating duration of estrus. In fact, from experience gained during the course of these studies, it became apparent that the ability of the observer to define estrus is far more important than the observations made more frequently than every 6 hr. The mean duration of estrus of 11.9 hr., as shown in Table 1, is about 5 hr. shorter than the average values reported by investigators from the temperate areas of the world (2, 17, 29) and agrees rather closely with the limited data from the tropics and subtropics (1, 10, 13, 24). It appears that environmental factors during the course of these studies had a depressing effect on the expression and duration of estrus. Although the feeding program, particularly the forages, was not considered to be optimum, the conditions that prevailed during the course of this experiment are rather typical of those on most of the good commercial dairy farms in the Gulf Coast region. Thus, it appears that the explanation for shorter estrous periods in this region is a complicated one, involving the possible interaction of nutrition, parasites, climate, and endocrine gland performances in the cow.

Some diurnal variation in onset of estrus was found with 28.6, 21.7, 23.6, and 26.1% of the estrous periods being initiated at 0600, 1200, 1800, and 2400 hr., respectively. Comparing night to day, it was found that 54.8% of the estrous periods began during the night, whereas 45.2% began during the day. These

TABLE 1

Duration of estrus in the L.S.U. dairy herd

Total number of animals	270
Total estrous periods observed	1,460
Average duration of estrus (hr.)	11.9
Median (hr.)	12.0
Mode (hr.)	12.2
Standard deviation (hr.)	6.1
Range (hr.)	2–46

[3] Trademark, Hormone Chemistry Laboratory, Houston, Texas.

results emphasize the importance of the early morning (0600 hr.) observational period, especially if under practical dairy farm conditions it is not deemed feasible to include a midnight heat check.

Since approximately one-half of the estrous periods were less than 11.9 hr. in duration, the question arises as to how often heat checks should be made on a dairy herd under such environmental conditions as reported herein. Using the 0600 and 1800 hr. observations as a basis for comparison, it was found that an additional observation period at 1200 hr. increased efficiency by 10.0%. The fourth observational period gave an increase in efficiency over the twice-daily heat checks of 19.9%. Such improvement in breeding efficiency would certainly justify checking for estrus more than twice daily, especially during the heavy breeding season.

Also included in the 1,460 estrous periods shown in Table 1 are 147 periods during which the cows and heifers did not stand when mounted by their herdmates. Rectal palpation of these animals revealed that the vast majority had ovarian activity, that is, follicular development. One hundred of these cows were bred, resulting in 45 pregnancies. Such breeding efficiency compares favorably with the 46% breeding efficiency for all first and second inseminations shown in Table 2. These results confirm those of Trimberger (28), who bred cows during nonstanding estrus as efficiently as those during standing estrus.

TABLE 2

Relationships among time of breeding, age, and rate of pregnancy

Hour of breeding	Age [a]	Services [b]	Pregnancy [c]
		(No.)	*(%)*
0–6	A	3	33
	B	7	14
	C	0	—
	Total for all ages	10	20[d]
7–12	A	35	60
	B	69	57
	C	14	36
	Total for all ages	118	55[d]
13–18	A	44	39
	B	104	51
	C	28	36
	Total for all ages	176	46
19–24	A	32	47
	B	62	31
	C	19	47
	Total for all ages	113	38[e]
	A	114	47
	B	242	46
	C	61	41
	Total for all hours and ages	417	46

[a] Age was partitioned and designated as follows: A—nulliparous animals (heifers); B—cows with one to four lactations; C—cows with five or more lactations.

[b] First and second services.

[c] Pregnancy determined by rectal palpation.

[d] Confidence limits outside of group mean at 10% level of probability.

[e] Confidence limits outside of group mean at 20% level of probability.

Subclass confidence limit = mean $\pm$ ^{tn} 0.01 or 0.05 $\sqrt{\frac{p(1-p)}{n}}$.

Age was found to have an effect on the duration of estrus, with nulliparous animals having a significantly ($P < 0.01$) longer period than parous cows with 1–4 lactations. However, the difference between nulliparous animals and cows with five or more lactations was less ($P < 0.05$). Such results do not agree with those of Trimberger (27), who reported that older animals had longer estrous periods. The variation in results for cows and heifers in this experiment is, perhaps, due to the fact that the lactating animals could not be observed so closely as the heifers, because of the 2 or 3 hr. confinement each day necessary for grain feeding and milking. Also, the stress of lactation under these environmental conditions may have been a factor in decreasing the duration of estrus among the parous animals.

Some monthly variation was found in the duration of estrus, with the means for April (10.6 hr.) and May (10.8 hr.) significantly shorter ($P < 0.01$) than the means for September (13.9 hr.) and December (13.7 hr.). None of these differences reflected a seasonal pattern.

Postconception estrus occurred in 14.4% of the cows and represented 3.4% of all estrous periods. These results generally agree with those of Erb and Morrison (9), despite the greater length of the study reported by the latter workers. Rectal palpation of the cows exhibiting postconception estrus revealed little or no ovarian activity. Such estrous periods were classified as false estrus, that is, psychological, without physiological estrus.

The average length of the interval to first postpartum estrus for 169 cows was 38.4 days, with a standard deviation of 29.5 days. This is a somewhat shorter period than previously reported (9, 11). This lack of agreement may have been due to the different frequencies of observation for estrus among studies. Also, some of the cows that exhibited anestrus for periods longer than 90 days postpartum in the studies reported herein were given hormonal treatment, thereby preventing most of the extremely long intervals.

Estrous cycle. A total of 1,030 estrous cycles ranging from 2–146 days in length were observed. Of all cycles, 69.3% were in the 16–24 day range and were classified empirically as normal within this experiment, since they were considered to characterize the estrous cycles of the experimental animals. Parameters calculated on the 709 normal cycles were as follows: mean, 20.7 days; mode, 21 days; and standard deviation, 2.1 days. Cycles less than 16 days in length were thought to be the result of cystic ovarian conditions or lack of normal cyclic behavior shortly after calving. Those approximating 40 to 60 days were considered to be multiples of normal cycles which were not observed. Such results for normal estrous cycles compare favorably with the literature (1, 3, 7, 20, 22, 28), thereby indicating that the environmental conditions of this experiment had little or no effect on the periodicity of estrus.

Age was found to have a significant effect ($P < 0.05$) on the length of normal estrous cycle length. The mean length for nulliparous animals was 20.4 days, as compared to 20.9 days for cows with 1–4 lactations. Cows with five or more lactations had cycles of intermediate length of 20.7 days. The modal value of 21 days

for heifers, as compared with a modal value of 22 days for cows, agrees with the literature as summarized by Asdell (3).

Ovulation time. The average time of ovulation for 51 animals was 12.4 hr. after the end of estrus, which is in close agreement with most results reported from the temperate (2, 3, 17, 28) and subtropical (10) areas of the world. Heifers ovulated sooner (10.8 hr.) than did parous cows (12.9 hr.). However, numbers were small in the heifer age-group.

Factors affecting breeding efficiency. The effects of time of insemination on level of fertility are summarized in Table 2. It should be emphasized that, as pointed out by Erb and Holtz (8), purebred data such as reported herein can be expected to show lower efficiencies than the average herd, because greater emphasis is placed on progeny from favored brood cows and outstanding proved bulls, and the owner usually retains such animals longer than the average dairyman, even though fertility may be low. As will be noted in Table 2, the fertility was highest for inseminations made from 7–12 hr. after the onset of estrus. This range represented the period from the middle to the end of estrous periods in this study.

Estrous cycle length was found to affect fertility ($P < 0.05$) only when the cycle preceding service was less than 17 days in length. When the effect of time of service was held constant, a highly significant ($P < 0.01$, degrees of freedom = 301) correlation of .18 was found between estrous cycle length and fertility. Such results compare favorably with those of Erb and Ehlers (7) and of Trimberger (28).

Level of fertility was found to vary with age, as shown in Table 2, which agrees well with the majority of previous investigators (8, 24, 28). Although the oldest age-group was much smaller than either of the other two groups, its low fertility undoubtedly contributed to the size of the highly significant ($P < 0.01$) partial correlation coefficient of 0.26, effects of level of milk production held constant.

As shown in Table 3, anestrus remained a problem in this herd, despite the more frequent observations for estrus. The value of 34.4% for anestrous cows is somewhat higher than most values in the literature (11, 28). However, comparison is difficult, due to the variation in methodology among investigators. The 10.6% incidence of missed estrous periods is low, but would have increased to 30.5% if observations for estrus had been made twice daily, as is generally practiced by most dairymen. If standing estrus had been demanded in this experiment, then 10.1% additional estrous periods would have been missed, thereby increasing the incidence of anestrous periods to 40.6%. It should be emphasized

TABLE 3

Incidence of anestrus by cows, breeds, and estrous intervals

Breed	Total cows	Anestrus cows		Total intervals	Anestrus intervals	
	(No.)	*(No.)*	*(%)*	*(No.)*	*(No.)*	*(%)*
Holstein	151	52	34.4	768	82	10.7
Jersey	95	31	32.6	572	57	10.0
Sindhi-Holstein	24	10	41.7	120	16	13.3
Totals or Av.	270	93	34.4	1,460	155	10.6

that clinical anestrus such as considered in this study includes both physiological anestrus and anestrus resulting from managerial practices.

Thyroidal activity. The mean monthly PBI values for 26 cows are given in Table 4, along with certain temperature and dew-point data. It will be noted that the monthly variations in PBI values closely paralleled the variations in climatic data. Such trends are in agreement with those reported for dairy bulls by Griffith (12) at this station and by Volcani (9) in Israel. Since there was no apparent seasonality of anestrus during the course of this experiment, it would appear that thyroidal activity by itself had no seasonal relationship with the incidence of anestrus. Consequently, since it has been shown that climatic conditions such as those shown in Table 4 place considerable stress on dairy cattle of the European breeds (12, 14), such results suggest that the expression of estrus is protected from certain changes in the environment by the interplay of various endocrine glands, especially the thyroid, ovary, pituitary, and the adrenal.

CONCLUSIONS

From these results and those in the literature, the following conclusions appear justified:

The duration of estrus of dairy cattle in the tropics and subtropics is of such short duration as to require more frequent observations for estrus than twice daily.

Cattle exhibiting such estrous periods as reported herein apparently should be bred 7–12 hr. after the onset of estrus. However, more research is needed on timing of service before this recommendation can be made unequivocally.

There appears to be no practical reason for delaying service because of estrous cycle irregularity if the cycle is at least 17 days in length and the cow is otherwise normal.

Anestrus is a breeding problem of considerable consequence. However, man-

TABLE 4

Relationship between mean monthly PBI levels for 1957 and certain weather data

Month	PBI [a]		Temperature [b]			Dew-point [c]		
						Temperature		Time above 70° F.
	Mean	S.D. [d]	Av. daily min.	Av. daily max.	Days above 90° F.	Mean	S.D.	
	—(μg. %)—		—(°F.)—			—(°F.)—		(%)
March	8.4	2.3	50	71	0	....	.....	.....
April	8.8	0.8	59	79	0	55	11.0	4.3
May	7.3	1.2	66	86	10	63	7.0	18.2
June	5.8	1.0	72	90	19	70	4.1	69.1
July	4.2	1.1	74	94	29	72	2.7	88.0
August	3.8	0.6	72	92	27	72	3.2	81.8
September	4.8	0.7	68	84	9	66	7.1	40.8
October	4.9	1.2	54	76	0	55	12.1	8.8
November	6.6	0.9	51	70	0	....	.....	.....
December	8.0	1.0	43	67	0	....	.....	.....

[a] PBI values from Johnston *et al.* (15).
[b] Temperature data for 1957 for Baton Rouge, La., U. S. Weather Bureau.
[c] Dew-point data (1950-54 averages) from Johnston *et al.* (14).
[d] Standard deviations should be preceded by ±.

agerial practices rather than physiological dysfunction appear to be the reason for most clinical anestrus. The role of nutrition, the thyroid, and climate in the problem of anestrus needs to be more definitely established.

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TECHNICAL NOTES

SIMPLIFICATION OF STANDARD METHODS FOR SALT ANALYSIS IN CHEESE

The salt analysis of cheese as recommeded by Wilster *et al.* (4) and by the Association of Official Agricultural Chemists (1) as standard procedures are adaptions of the Volhard technique for halide analysis in acid media. In its latter stages these procedures are somewhat time-consuming and cumbersome, due to the necessity for either a decantation or filtration step.

Essentially, the Volhard method involves the quantitative precipitation of chloride as silver chloride by the addition of a known but excess quantity of silver nitrate. The excess silver ions are then back-titrated with thiocyanate, using ferric ammonium sulfate as the indicator.

A difficulty exists due to the fact that silver thiocyanate is less soluble than the silver chloride precipitate. At the end-point any excess of thiocyanate will tend to solubilize silver chloride in the following manner (3):

$$AgCl + CNS^- \rightleftharpoons Ag\,CNS + Cl^-$$

Equilibrium will be established when the following relationship exists:

$$\frac{[Cl^-]}{[CNS^-]} = \frac{S_{AgCl}}{S_{AgCNS}} = \frac{1.12 \times 10^{-10}}{7 \times 10^{-13}} = 160$$

Therefore, unless silver chloride is prevented from interfering during the back titration with thiocyanate an indistinct end-point occurs. One method is to isolate the soluble silver ions from the insoluble silver chloride. Wilster *et al.* (4) obtained their separation by several decantations, whereas the A.O.A.C. method requires a filtration step and, after washing, removal of an aliquot portion of the filtrate for titration. Either of these standard procedures is needlessly elaborate. Nitrobenzene, as shown by Caldwell and Moyer (2), may be effectively employed to coat the silver chloride precipitate, preventing further participation during titration. This principle has been found to be applicable to the salt analysis of cheese.

In the modification, the A.O.A.C. procedure is followed through the digestion step. That is, to 3 g. of cheese, 25 ml. of tenth-normal silver nitrate, 10 ml. of nitric acid, and 50 ml. of water are added. Digestion is accomplished by boiling this mixture with three 5-ml. portions of 5% potassium permanganate. Then, after cooling the digest to room temperature, 2 ml. of nitrobenzene and 2 ml. of ferric ammonium sulfate are added with immersion pipettes and the entire contents in the flask shaken and titrated directly with tenth-normal potassium thiocyanate to a brick-red end-point. The normal steps of decantation or filtration, as discussed above, are thereby eliminated.

Salt analysis of many cheeses (Table 1), comparing this modification and the A.O.A.C. method, indicates that accuracy is not impaired. The Cheddar cheeses range in age from 1 mo. to approximately 2 yr. This modification has been used routinely in this laboratory for the past 4 yr. by both the staff and students. All have found the technique to be both simple and rapid, with a precise end-point. Nitrobenzene like many chemicals is toxic, and although no difficulties in its use have been experienced by this laboratory, proper precautions should be used during handling.

TABLE 1

Analysis of cheese for salt by A.O.A.C. procedure and by the nitrobenzene modification

Type of cheese	A.O.A.C.	Nitrobenzene
	(%)	
Natural Cheddar		
Ripened for 1–2 mo.	1.54	1.54
	1.55	1.55
	1.57	1.57
	1.57	1.55
	1.59	1.61
	1.61	1.59
	1.62	1.61
	1.63	1.63
	1.66	1.66
	1.69	1.72
	2.00	2.01
Ripened for 10–24 mo.	1.31	1.35
	1.80	1.85
	2.09	2.14
	2.16	2.14
	2.20	2.22
	2.21	2.19
	2.28	2.28
Processed		
Cheddar	1.83	1.88
Smoked Cheddar	0.99	1.04
Smoked Cheddar	0.97	1.01
Gruyère	1.97	1.98
Gruyère	1.05	1.01

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MECHANICAL DRAINING, INOCULATING, AND HOOPING OF BLUE MOLD CHEESE CURD [1]

Mechanization of the various operations in cheese-making has been attempted by numerous workers. Wilkowske and Fouts (5) devised a continuous fermentation apparatus for producing cheese starter. Harper and Seiberling (2) patented a process and equipment for continuous setting and cutting of cheese curd. Walter, Sadler, and Mitchell (4), Leber and others (3), working independently, shortened and mechanized the cheddaring and hooping operations for Cheddar cheese. This report deals with an apparatus for continuously draining, inoculating, and hooping Blue Cheese curd.

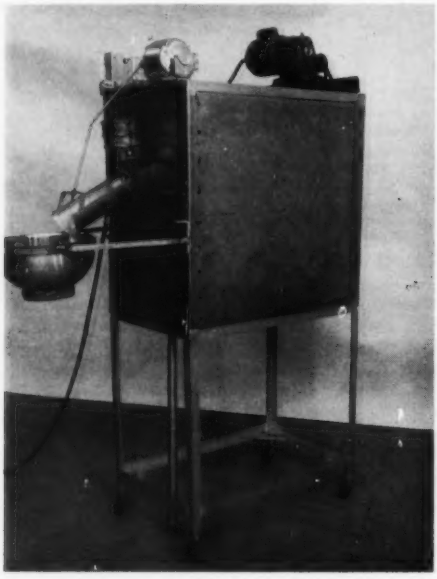

FIG. 1. The hooping machine is compact, portable, and enclosed. The side panels and exterior parts were designed for easy removal, to aid in cleaning operations.

In our experiments, the milk was set in 300-gal. vats, cut and firmed by the Lane-Hammer process (1). The agitated mixture of curd and whey was then pumped into a rotating perforated drum where the whey was separated from the curd. This drum was inclined 12 degrees toward the outlet. An inner spiral was not needed to push the curd through the drum. The curd dropped from the drum on to an inclined vibrating chute, above which was mounted a standard 40-mesh sieve filled with mold powder. Vibration of the chute delivered an even flow of mold powder on to the curd as it passed into a hopper. The funnel-shaped hopper was equipped with a quick-acting gate valve through which the inoculated curd dropped directly into the hoops. The quick-acting valve interrupted the flow of curd briefly while inserting empty hoops and removing filled hoops.

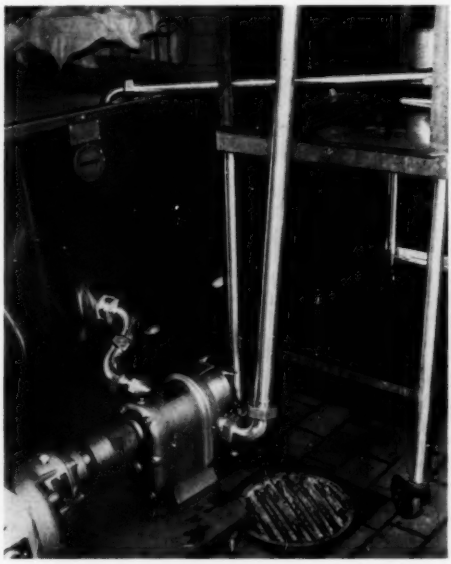

FIG. 2. A positive pump moves the curd and whey mixture from the vat to the perforated drum. The curd was not damaged by pumping as long as the mixture was at least 50% whey by volume, at a flow rate of 20 gal. per minute through 1½-in. sanitary pipe.

Hoops were filled at the rate of 2 to 2.5 per

[1] Technical Contribution No. 307, South Carolina Agricultural Experiment Station, Clemson College, Clemson, S. C.

minute in these trials. The rate could be increased by enlarging the drainage area in the perforated drum and increasing the pump speed. Moisture content of the cheese was controlled very accurately by adjusting the size of cut and whey acidity. In carefully controlled manual operation, moisture content of individual wheels in a lot varied plus or minus 1.0%. This deviation was reduced to plus or minus 0.25% in the mechanical process, with similar improvement in uniformity of cheese quality. Manual handling of the curd was eliminated.

The goal of complete mechanization of cheesemaking is not yet accomplished. However, exploitation of recent developments in curd-handling processes should significantly reduce production labor costs, increase capacity of present equipment, and improve uniformity of cheese. Serious thought and research should be devoted to developing accelerated, continuous procedures for cheese-curing. The traditional barrier of curing cheese in specified sizes and shapes might be ignored and thus utilize well-recognized continuous fermentation techniques developed in other industries. As an example, Blue Cheese curd suspended in a liquid or spread in thin layers could be molded in a fraction of the 4 to 6 wk. required in the usual process. Pressing into desired shapes could then be accomplished on the finished cheese.

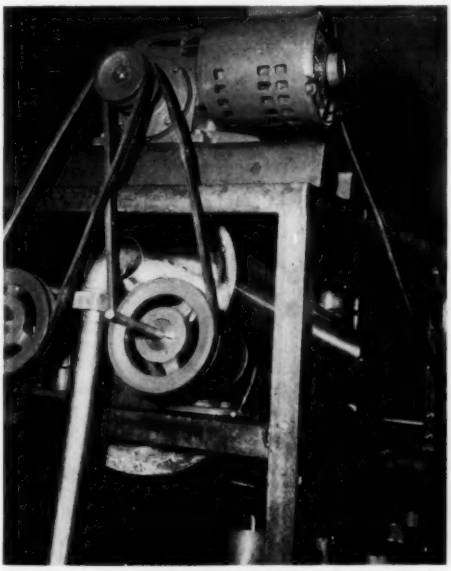

FIG. 3. The entrance of the perforated drum was cone-shaped, to prevent curd from falling out. The drum was inclined 12 degrees to aid forward motion of the curd. The drum rotated at about 20 r.p.m., with a friction drive. The trough under the drum collected the whey, which was piped away or recirculated as needed. Curd was removed from the drum by four baffles in the outlet end.

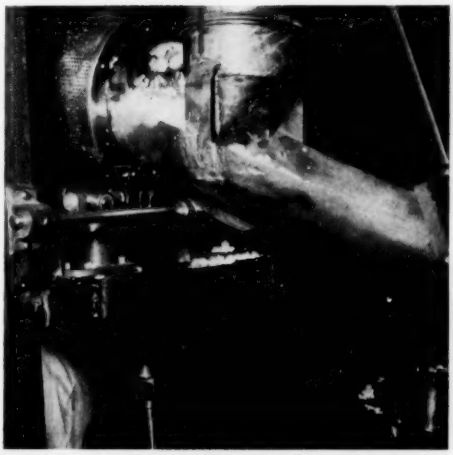

FIG. 4. Mold powder in the sieve over the vibrating chute was dusted lightly on to the curd as it dropped to the hopper. The rate of inoculation was varied by the pore size of the sieve.

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INFLUENCE OF TEMPERATURE HISTORY ON THE RESPONSE OF PSYCHROPHILES TO DIFFERENT INCUBATION TEMPERATURES

The routine bacteriological technique of incubating cultures at temperatures suitable for growth followed by refrigerating to terminate growth presents a frustrating problem to the in-

vestigator of psychrophilic bacteria. With these organisms, one is faced with a sequence of two actual incubation periods—the first suitable for rapid proliferation, and the second suitable for high viable counts (1). This sequence of incubations might influence the subsequent behavior of the culture. Sherman and Cameron (3) have shown that coliform bacteria lost their viability when transferred from 45 to 10° C; whereas, cultures originally grown at 10° C. retained their viability when subcultured at 45° C. Olson and Schultze (2) studied psychrophiles and reported that the relative ability of their cultures to oxidize glucose at three different temperatures was a function of the temperature at which the cultures previously had been grown.

To study the possibility that propagation temperature history might influence the behavior of subcultures, a series of simple trials was performed using a typical psychrophile, a proteolytic strain of *Pseudomonas fluorescens* isolated from cottage cheese. The culture was grown in nutrient broth at 37° C. (near its maximum), at 20°, and at 5° (near its minimum). Growth was measured turbidimetrically at regular intervals during a 48-hr. period, and growth curves were plotted. By repeating these trials many times, it was possible to predict with some accuracy the pattern of the culture's development at each temperature. It was then possible to plan an inoculation schedule which would provide at one given time a sample from each mother culture which contained approximately the same total number of cells at approximately the same physiological stage of growth. These samples were used as inocula into flasks of nutrient broth which were incubated at 37, 20, and 5° C., respectively, and the resulting nine growth curves (three at each temperature from each of three mother cultures) were plotted. These curves are shown in Figure 1.

The incubation temperature of the mother culture had a definite effect on the response of the subcultures to their subsequent incubation temperatures. There was a better response at 20° C. when subcultures were taken from 5 and 20° C. than when taken from 37° C. Furthermore, incubation at 5° favored subculturing at 5°, whereas incubation at 37° favored subculturing at 37°. These results would be expected from theoretical considerations and illustrate the possible discrepancies which might result from studies on psychrophiles that have been "stored" at refrigeration temperatures in a viable state. It is beyond the scope of this note to delve into the polemics of adaptation or selection or mutation as an explanatory mechanism for this phenomenon—but it is worth while to mention that studies on psychrophiles must take their temperature history into consideration, particularly if growth ability at different temperatures is to be investigated.

A further development of this problem was the possibility that pre-exposure to refrigeration temperatures (such as might be employed in a dairy plant) might accelerate the growth of bacteria at other refrigeration temperatures (e.g., in household refrigerators). This was studied by dispensing a lot of freshly pasteurized milk into sterile containers, half of which were kept at 8° C. and the other half at 2° C. At daily intervals for several days, two containers from the 8° box were exchanged with two from the 2° box—eventually yielding a series of samples which had been subjected to the same two temperatures for comparable lengths of time, and which differed from each other only in the initial temperature to which they had been exposed. Standard plate counts were performed on all samples, and the results are presented in Table 1.

TABLE 1

Influence of sequence of exposure to two refrigeration temperatures on the standard plate count of pasteurized milk

Initial temperature	Period at initial temperature	Second temperature	Period at second temperature	Standard plate count
(° C.)	*(days)*	*(° C.)*	*(days)*	*(per ml.)*
8	5	..	..	5,900,000
2	5	..	..	8,900
8	4	2	1	560,000
2	1	8	4	790,000
8	3	2	2	60,000
2	2	8	3	380,000
8	2	2	3	41,000
2	3	8	2	184,000
8	1	2	4	54,000
2	4	8	1	124,000

It is possible, according to these data, that differences in the initial refrigeration temperature have an influence on the ultimate count. This influence is certainly not as important as that of the actual environmental temperature, and from a bacteriological point of view is relatively insignificant, the counts being at the maximum only two or three generation times apart. However, from the legal aspect and perhaps from the keeping time aspect, this influence might be important. The observation that exposure to the lower temperature (2°) followed by exposure to the higher (8°) resulted in every case in a greater count than did exposure to the higher temperature followed by exposure to the lower, might be of practical significance if substantiated. A great deal of further study under laboratory and plant conditions is indicated in this field.

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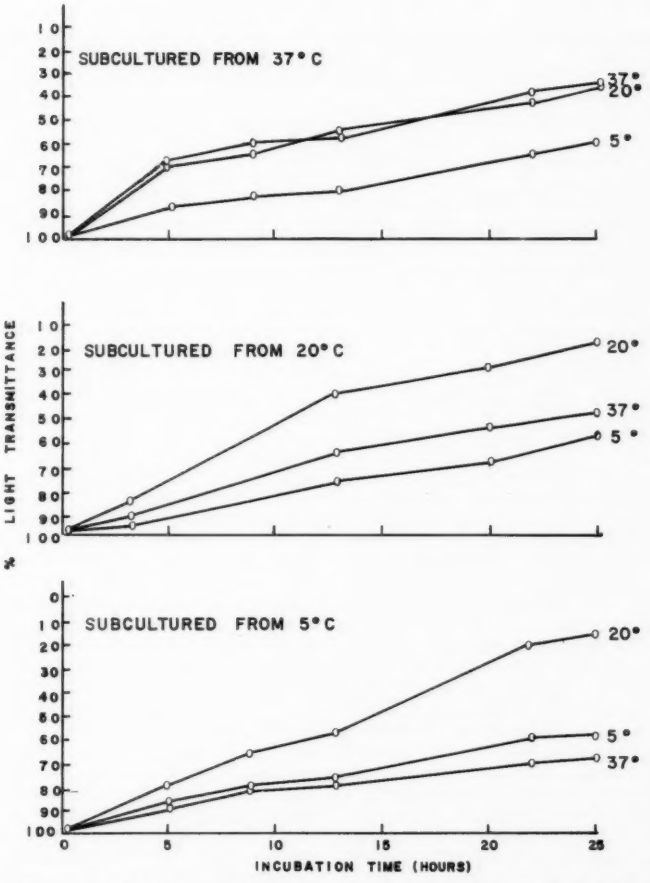

FIG. 1. Growth of *Pseudomonas fluorescens* (measured turbidimetrically in nutrient broth) at three different temperatures as influenced by incubation temperature of mother culture.

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INFLUENCE OF BACTERIAL INTERACTION ON RESAZURIN REDUCTION TIMES

One of the major factors contributing to the inaccuracy of dye-reduction tests as a means of evaluating bacteriological quality of raw milk is the variation in reducing ability of different bacteria. It has been well established by Garvie and Rowlands (3) and others that different pure cultures reduce methylene blue or resazurin at markedly different rates, and this phenomenon is commonly indicated as being responsible for discrepancies between dye reduction and other quality tests. A further source of error might be in the interaction among the normal flora of raw milk, resulting either in an accelerated or retarded rate of dye reduction. Such an associative action, observed during qualty tests on egg pulp (4), has also been reported in the reduction of methylene blue in milk by Davis and Lines (2), but their results are based on speculative rather than direct experimental evidence. If there is a true interaction among

TABLE 1

Resazurin reduction times in minutes of pure and mixed bacterial cultures in reconstituted nonfat dry milk solids

Culture	Optical density of inoculum		
	1.9	0.5	0.2
Pseudomonas fluorescens No. 169	60	105	.....
Pseudomonas fluorescens No. 92	90	105	.....
Pseudomonas fluorescens No. 169 + *Pseudomonas fluorescens* No. 92	45	105	.....
Pseudomonas fluorescens No. 169	60	105	300
Bacillus subtilis	15	90	225
Pseudomonas fluorescens No. 169 + *Bacillus subtilis*	15	45	120
Pseudomonas fluorescens No. 92	90	105	300
Bacillus subtilis	15	90	225
Pseudomonas fluorescens No. 92 + *Bacillus subtilis*	15	45	135
Streptococcus lactis	15	15	.....
Streptococcus lactis + *Pseudomonas fluorescens* No. 92	15	15	.....
Streptococcus lactis + *Bacillus subtilis*	15	15	.....

the organisms of raw milk, this phenomenon should be investigated and its significance evaluated.

During the course of a study on the interaction of bacteria, it was found that such typical species as *Streptococcus lactis, Pseudomonas fluorescens,* and *Bacillus subtilis,* isolated from raw milk, exerted an influence on each other's ability to reduce resazurin when grown in reconstituted nonfat dry milk solids. A typical experiment is depicted in Table 1. The inocula for this trial were grown in a suitable nutrient broth, were centrifuged and washed in saline, and were resuspended in saline to one of three concentrations. The heaviest suspension, having an optical density of 1.9, contained approximately 100,000,000 organisms per milliliter. The other concentrations were adjusted to optical densities of 0.5 (approximately 1,000,000 per milliliter) and 0.2 (approximately 100,000 per milliliter). Aliquots of these saline suspensions were added to the reconstituted milk to yield milk samples representing three arbitrary grades of bacterial quality, and resazurin tests were performed on the samples according to standard procedures (1). Where interaction was to be studied, half of the usual inoculum was used for each culture, the total ultimate bacterial concentration being identical to the pure culture controls.

As expected, the speed of dye reduction was a function both of the total population and of the type of culture inoculated. Furthermore, there was an acceleration of reduction when combinations of cultures were involved. Though this latter influence was not as marked as the two former ones, in certain instances the interaction assumed a major significance. The demonstration of synergism itself depended on inoculum size and was masked by very heavy inocula in some cases (*B. subtilis–Pseudomonas* sp.) or became undemonstrable by very light inocula in other cases (*Pseudomonas–Pseudomonas*). The strong reducing capability of *S. lactis* overshadowed any interactions between this culture and the others at the concentrations employed. Since raw milk, especially with bulk tanks, can be expected to have fewer *S. lactis* and more psychrophiles, synergistic effects of these combinations are currently being studied under conditions more closely approximating those found in the field. It might be important to note that the most marked interactions were evident between *B. subtilis* and *P. fluorescens* in milks that would be considered to be of good quality. If this phenomenon is universal during routine examination of raw milk samples, then it is possible that a producer is being penalized not so much because the milk he is selling contains many organisms, or even because they are fast dye-reducers, but rather because they are synergists instead of being antagonists.

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OUR INDUSTRY TODAY

ECONOMICS OF VARIOUS METHODS OF HARVESTING AND UTILIZING FORAGES[1]

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Whether it pays to use one combination of forage harvesting and feeding methods over another depends on the availability of cropland; on the intensity of the livestock program; on available buildings, feed storage facilities, equipment, and capital; on the labor supply; on the quantity and quality of feed nutrients preserved by different methods, and on other factors. Dairymen who expect to make changes in their dairy housing or size of herd need to consider these possibilities when selecting new forage methods or equipment. Personal preferences often play an important role in these choices.

The problems encountered in a study of forage economics are complex and can not be studied in isolation. To determine the economic feasibility of a new harvesting or storage method, it is essential that it be related to the whole farm business. What effect will the method have on labor peaks, on investments, expenses, and net income? Are there other investment alternatives that will result in greater returns? The fact that farms vary greatly in size and resources suggests that there is no one best forage system for all farms. A system that is profitable in one region may be entirely inappropriate elsewhere, because of differences in climate, farm resources, and cost-price relationships.

It is the purpose of this article to outline a few alternative forage-harvesting, storage, and feeding methods currently used, and to discuss the effect of their adoption on investments, labor requirements, expenses, and net income under conditions similar to those in Michigan. These alternatives have been tested for farms differing in size and resources. Farms with 25–30 cows and 60 cows were selected for study. The budgeting technique was used in calculating receipts, expenses, and net income for the various alternatives. Prices used were largely those representative of 1957 price relationships.

SELECTING APPROPRIATE RELATIONSHIPS

An agricultural economist, in developing the most profitable forage production and utilization program for a particular farm, needs three general types of information (15). First, he must know what physical and financial resources are available for the farm; secondly, the prices of the various inputs and outputs, and thirdly, the physical input-output functional relationships and rates of substitution that exist for the farm. For this last type of information, the economist largely must depend on the results of experimentation by agronomists, animal nutritionists, and agricultural engineers.

The reliability of the answers we obtain in budgeting various forage harvesting and utilization methods depends on the appropriateness of the input-output relationships used. It is more important that these input-output data represent the relative rather than the absolute relationships for the alternative tested. We, in agricultural economics, often find some of the experimental data relating to forage production and utilization inappropriate for economic analysis (7, 17, 23). The data have three shortcomings: *(1)* Results are based on controlled experiments where the conditions for production and levels of management involved are often so unusual that inferences relating to real farm situations are unrealistic, *(2)* the form in which the results are reported makes economic interpretation difficult (for example, results reported as T.D.N. with no figures provided on tons, pounds, or acres of forage fed), and *(3)* in the case of feeding trials, results are often reported for only a portion of the barn or pasture feeding period.

Estimating quantities and qualities of forage preserved. The profitability of investing in improved hay and silage harvesting, storage and feeding equipment depends to a large extent on the changes in quantities and qualities of feed nutrients preserved, and in changes in expenses and returns for the new operations.

Quantities preserved for feeding. We have estimated the percentages of dry matter preserved from an acre of alfalfa-brome grass as 68% for field-cured, baled hay, 78% for hay that was crimped or crushed and then baled, and 68 to 88% for silage (Table 1). The last two figures are for silage stored in uncovered bunker and gas-tight silos (19). The percentage for a large concrete stave silo of the same size as the gas-tight silo would be 85. It appears possible to preserve at least 78% of the feed nutrients in silage stored in bunker silos by using a plastic cover. The percent-

[1] Presented at the joint meeting of the American Grassland Council and the American Dairy Science Association at North Carolina State College, Raleigh, June, 1958.

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TABLE 1

Total digestible nutrients preserved for feeding when different harvesting and storage methods are used, one acre of highly productive alfalfa-brome grass stand [a]

Harvesting and storage method	Nutrients preserved	Tons of forage preserved	
		First crop	Second crop
	Hay(%)	Hay	Hay
Field-cured, baled	68	2.1	1.2
Crushed or crimped and baled	78	2.5	1.2
Chopped and barn-dried	78	2.5	1.2
Direct-cut silage	Silage [b]	Silage	Hay
Bunker	68	5.8	1.2
Bunker and plastic cover	78	6.7	1.2
Wilted silage			
Concrete stave, 14 ft. diameter	82	7.0	1.2
Concrete stave, 20 ft. diameter	85	7.3	1.2
Glass lined, 20 ft. diameter	88	7.6	1.2

[a] Produced on moderately productive soils in south-central Michigan.

[b] Storage losses will vary considerably with the type and size of silo, and with moisture content and preservative used. The per cent of nutrients preserved in tower silos is based on wilting the forage to 65–70% for the concrete stave silo and to 55–60% for the gas-tight silo. A plastic cap is assumed for the concrete stave silo.

ages and yield data are based on recent research studies by Shepherd *et al.* (21), Trimberger *et al.* (24), and Hoglund (8).

Dairy farmers have been encouraged to purchase direct-cut forage harvesting equipment because of the reduction in labor requirements and weather losses. On the basis of high seepage losses reported by experimenters (1, 2, 11, 14) and farmer experiences, the direct-cut method does not appear desirable when silage is stored in tower silos 16 or more feet in diameter and 40 or more feet in height, unless a conditioner is added. Seepage losses of 12% of the dry matter ensiled are not unusual when no conditioner is used.

Experiences of one of Michigan's more successful dairy farmers point out that a better method is to wilt the material when weather conditions permit, but to be prepared to add sufficient ground corn and cob meal or other conditioners to absorb the excess moisture in the forage when wilting is not feasible. A continuous silage harvesting operation and the preservation of larger quantities of high-quality forage result. The more successful dairy farmers harvest 25 or more tons of silage per hour with a well-organized combination of tractors, equipment, and men.

As dairy farms become larger, and farm labor becomes more expensive and, possibly, unionized, it may become economically feasible to depart from the customary feeding of grain according to individual cows' milk production. The effect on milk production and net returns of feeding most or all of the grain ration as part of the silage feeding needs investigation. If this practice were profitable, then the direct-cut forage harvesting method plus the use of a conditioner to soak up excess moisture could well be an attractive alternative.

Potential feeding value of forage. Increased opportunities of mechanizing feeding operations provide additional advantages for silage over hay. This becomes an important consideration in increasing the effectiveness of labor on the larger dairy farms, where such investments are economically feasible. Although there is no general agreement, many forage experiments (6, 10, 13, 16, 21) indicate that good-quality silage can make up a high proportion of the ration without loss in milk production or body weight. Some of these experiments show the need for the inclusion of some minimum quantity of hay for best results. Three levels of silage feeding were budgeted for the 60-cow farm. The pounds of hay and silage fed were calculated on the basis of T.D.N., equaling 0.20 for silage and 0.50 for hay. These combinations ranged from a low level of 25 lb. of silage and 30 lb. of hay to a high of 80 lb. of silage and 8 lb. of hay fed per cow daily.

Forage fed daily to 1,200-lb. Holstein cow (lb.)	*Level of silage feeding:*		
	Low	*Medium*	*High*
Silage	25	50	80
Hay	30	20	8

We have estimated equal milk production when cows are fed silage at low, medium, and high rates, when the grain ration is adjusted for differences in protein and T.D.N. in the total hay and silage ration fed. Grain feeding was calculated at the rate of 1–4 lb. of 3.5 test milk produced and milk sales per cow at 9,700 lb. per cow. We also estimated that alfalfa-brome grass silage stored in the three types of silos would have equal feeding value if appropriate practices accompanied the filling and storage of the material ensiled (19,

20). The gas-tight silo provides more flexibility than the other silos when different crops are harvested throughout the season.

Estimating carrying capacity for different pasture systems. Estimating the carrying capacity of an acre of forage when different pasture systems are used is much more difficult than determining hay or silage yields. Wide year-to-year and area-to-area variations in acres used per cow, or estimated T.D.N. production per acre, for alternative pasture systems as reported by pasture researchers (4, 5, 12), appear partly to be due to differences in manipulation of the stocking rate and supplementary feeding of forage and grain. Differences in available moisture during the growing season, length of growing season, crops used, and soil fertility also contributed to variations in results.

Results of pasture experiments at the Louisiana (22), Minnesota (5), Mississippi (18), and Wisconsin (12) stations showed savings of 18 to 60% in acres of pasture needed per cow when the newer pasture systems such as strip-grazing, green-chopping, and storage-feeding were used. These reductions are not of the magnitude often reported in farm magazines. A Utah (3) experiment comparing rotational grazing and green-chopping resulted in the same milk output per acre for both systems.

Because of the difficulties of using pasture research data for economic analysis, a Michigan farm study of grazing systems was started in 1954. A total of 60 different farms was included in this study. We have estimated, on the basis of this 4-yr. study (7, 8) and other research, that when good management techniques are applied to all systems, strip-grazing will increase carrying capacity by 15–20%, and green-chopping and storage-feeding by 25–35%, over improved grazing. These estimates are based on using alfalfa-brome grass for the improved-grazing and storage-feeding systems, and a combination of alfalfa-brome grass and Sudan grass for the strip-grazing and green-chopping programs. Improved grazing, contrasted to straight continuous grazing, is described as including some form of rotational grazing and the supplementary feeding of hay and/or silage when the pasture supply becomes short. It usually means using fewer acres of full-season pasture the early part of the grazing season.

SELECTING PROFITABLE FORAGE ALTERNATIVES

We selected two farm situations to test the economic consequences of using different combinations of forage equipment and methods, and alternative pasture systems. The first is a farm with 110 acres of cropland and 27 Holstein cows. This farm is typical of many 25–30 cow dairy farms in the Midwest. The labor force consists of the operator and his 16-year-old son. The cows are housed and milked in a stanchion barn. If this farmer spent $800, he could convert two horse stalls into stanchion room for five more cows.

The second farm has 200 acres of cropland and 18 acres of semipermanent grass-legume pasture. The labor force consists of the operator and a full-time hired man. This farm has undergone considerable change in organization and operation, largely as a result of improved technology. The milking herd has been increased from 35 to 60 as a result of a shift to a loose-housing system, pipeline milking, and other labor-saving equipment. Both farms have moderately productive cropland. For the purpose of this study, the operators are considered to have the managerial skill required to adopt any of the forage alternatives considered.

Alternatives for the 25–30 cow farm. We are starting out with a farm of limited crop-acres, a low investment in forage equipment, and a stanchion barn. Forage quality has been only fair, since all of the hay has been field-cured and custom-baled. A 90-ton silo has limited silage feeding to about 25 lb. per cow daily. This farmer, like many others, would like to improve on both the quantity and quality of forage preserved for feeding. He also feels the need for expanding herd size. An important problem is how to allocate limited funds among investments in new equipment, more cows, and other items of production.

Allocating limited capital. The first alternative tested was the allocation of $5,000 among investments in new forage equipment, in remodeling the barn, and in more cows. Should this farmer invest all of the $5,000 in forage equipment, or would he gain more if he first spent $800 for room for five more cows, added more cows, and invested the remainder for additional forage equipment?

Four alternative plans for investing the $5,000 were developed. Plan 1 provided for remodeling the barn, purchasing five additional cows, and investing the remaining $2,400 in a baler and hay conditioner. All hay would be baled, and corn silage for a small tower silo would continue to be custom-harvested. In the second plan, the barn would be remodeled and a baler and 16- by 40-ft. tower silo would be added. All grass and corn silage harvesting would be custom-hired. This would leave enough funds to purchase three cows. Plan 3 calls for investing in a 16- by 40-ft. tower silo and a barn-drier. Enough capital would be left to fix the barn and add four cows. If the fourth alternative were followed, this farmer would invest the entire $5,000 in a chopper, blower, and a silo. The operator would harvest all hay and silage with his own chopping equipment. A medium level of silage would be fed, if Plans 2, 3, or 4 were adopted.

This operator would increase net income by about $860 if he invested the $5,000 in the manner suggested in Plan 1, and $130 to $150

TABLE 2

Estimated changes in receipts, expenses, and net income when $5,000 additional capital is used to purchase different combinations of forage equipment and dairy cows (110-Acre dairy farm starting with 27 Holstein cows)

Item	Investment plan: 1	2	3	4
	($)			
Additional cows	5	3	4	.....
Changes in receipts				
Milk and cattle	+2,275	+1,365	+1,820	.....
Crop sales	.....	.....	.....	+187
Net change in receipts	+2,275	+1,365	+1,820	+187
Changes in expenses				
Fuel, oil, and custom-hire	− 443	− 339	+ 12	−578
Depreciation, repairs, and insurance on forage equipment	+ 392	+ 352	+ 224	+631
Miscellaneous dairy [a]	+ 230	+ 154	+ 266	.....
Hired labor	+ 100	+ 100	.....	+100
Feed and bedding	+ 835	+ 69	+ 312	−669
Interest on additional investment	+ 300	+ 300	+ 300	+300
Net change in expenses	+1,414	+ 636	+1,114	−216
Change in net income	+ 861	+ 729	+ 706	+403

[a] Includes depreciation and upkeep on additional barn investment, D.H.I.A., breeding, veterinary, and other expenses for the additional cows. Expenses for additional electricity used in operating barn-drier included for Plan 3.

less if he invested in fewer cows and an extra silo or barn-drier, as suggested in Plans 2 and 3 (Table 2). Plan 2 would add at least as much to net income as Plan 1, if two more cows were added at a later date. Investing all of the $5,000 in forage-chopping equipment and a silo would be a poor alternative for the short-run period, resulting in less than one-half as much gain in net income.

An $800 investment for a hay crimper or crusher is estimated to add $130 annually to net income, when as many as 30 acres of first-cutting hay are harvested. Additional investments needed to harvest and store all of the first-cutting forages for silage or barn-dried hay are not likely to be profitable on farms of this size.

Changing the pasture system. Would it pay the 30-cow dairy farmer who now follows an improved grazing program to adopt a strip-grazing or green-chopping system? We shall assume that this dairyman previously had made the investments specified in Plan 2. He owns a baler, but custom-hires all silage harvesting.

If this dairyman decided to strip-graze his pastures, he would need to invest no more than $250 in extra fencing. Feed purchases would be reduced by $435 as a result of a smaller acreage needed for grazing, and expenses for planting 12 acres of Sudan grass, and extra expenses for fencing and interest on additional investment, would add $75, leaving a net gain of $360 in favor of strip-grazing (Table 3). This would give the operator $2.30 a day for his efforts in moving fences and for the better management needed.

Should this dairyman decide to adopt a green-chopping system, and use the chopping equipment to harvest all of the silage produced, he would need to invest in a chopper, blower, direct-chop head, and feeding wagon, totalling $3,600. This would make it possible for him to chop all hay and silage crops pro-

TABLE 3

Estimated changes in investments, receipts, and expenses when a change is made in the pasture system (30-Cow dairy farm)

Item	Change from improved grazing to: Strip-grazing	Green-chopping
	($)	
Additional investment	+250	+3,600
Changes in receipts [a]		
Changes in expenses		
Fuel, oil, and custom-work	—	− 61
Depreciation, repairs, and insurance on forage equipment	—	+ 270
Fertilizer, lime, and seed	+ 42	+ 42
Fences	+ 25	− 48
Hired labor	—	—[b]
Feed	−435	− 620
Interest on additional investment	+ 8	+ 108
Net change in expenses	−360	− 309
Change in net income	+360	+ 309

[a] Same milk production per cow assumed for all systems.

[b] From 30 to 75 min. are usually required per day to chop and haul forages for a herd of this size. An individual dairyman will need to estimate the effect on net income of using this labor on other jobs compared to chopping and hauling forages to his cows.

duced on the farm. Net income would be increased by $309, or slightly less than for the strip-grazing system, which requires considerably less labor. If this farmer already owned field-chopping equipment, the green-chopping system would have been more profitable. The choice of one of these systems over the other would be determined largely by equipment on the farm, location of forage fields, and conflicts in the use of labor and equipment for other work. Strip-grazing usually requires less labor than green-chopping, and electric fences normally can be moved earlier in the morning or in the evening, when there are less conflicts with field work. These calculations do not include an annual charge based on the investment in an extension of the concrete feeding area, a step which is often necessary when a green-chopping forage system is added. Year-around storage-feeding and manure removal in a stanchion barn is not a particularly attractive alternative for this farmer.

Alternatives for the 60-cow farm. As herd size increases, so do the opportunities for increasing net income by investing in improved forage-harvesting, storage, and feed-handling equipment. When increased herd size is accompanied by a loose-housing system, additional forage alternatives are available for consideration. Up to a certain point, there are some economics of scale (increased size of operation) in harvesting, storing, and feeding forage crops. Annual storage costs per ton of silage stored in tower silos are reduced as diameter size is increased. Investments in forage chopping and storage equipment suitable for a 60-cow herd will average only 60% more than investments for a 30-cow herd.

Increase level of silage feeding. We shall assume a situation of low-silage feeding for the 60-cow farm. Present silage capacity is 120 tons. This farmer owns a baler but custom-hires all silage harvesting. Total investment in forage equipment is $8,000. How much will investments be increased, and how much will net income be changed, by adding a chopper, blower, self-unloading wagons, silo unloaders, mechanical feeders, and additional concrete stave tower silos, so that the level of silage feeding would be increased to *(1)* a medium and *(2)* a high level?

Investments would be increased by $8,700 in increasing silage storage capacity to a medium level (50 lb. per cow daily) and in mechanizing all operations to reduce labor requirements. To bring the silage feeding level to a maximum of 80 or more pounds per cow daily, would add $10,000 to investments. For the high silage situation, the baler would be traded in for chopping equipment, because of the small tonnage of hay harvested.

Changing to a medium or heavy rate of silage feeding would add considerably to annual charges for depreciation, repairs, insurance, and interest for the new investments. These increases would be more than offset by reductions in feed purchases. Net income would be increased by $673 if this farmer made the necessary investments for a medium-silage feeding rate and by $1,173 if he invested the extra $10,000 for the high-silage feeding program (Table 4). A 3% drop in milk production would cancel out the income advantages of the high rate of silage feeding. Less labor would be used in feeding the high-silage forage ration.

TABLE 4

Estimated changes in receipts, expenses, and net income resulting from an increased harvesting and feeding of silage
(60-Cow dairy farm)

Item	Change from low-silage, high-hay ration to:	
	Medium silage, medium hay	High silage, low hay
	($)	
Additional investment	8,700	10,000
Change in receipts[a]		
Change in expenses		
Fertilizer, lime, and seed	+ 68	+ 68
Fuel, oil, and custom-hire	− 273	+ 65
Depreciation, repairs, and insurance on forage equipment	+1,144	+1,002
Feed and bedding	−1,873	−2,608
Interest on additional investment	+ 261	+ 300
Net change in expenses	− 673	−1,173
Change in net income	+ 673	+1,173

[a] Assume same milk production per cow. No change in crop sales.

Investments in three types of silos. How much would net income be changed when a limit of $14,500 is placed on investments in different types of silos, other forage equipment, and cows? It is assumed that present cow numbers are 55 and the maximum number that could be handled by this two-man operation is 63. A 20- by 50-ft. concrete stave silo and unloader cost about $4,400, and a gas-tight silo of simliar size cost about $11,200 during 1957. A bunker silo of the same storage capacity, built of 2-in. tongue-and-groove planks cost $1,500 for the sides and an additional $1,200 for concrete floor and approaches, when the construction was hired. In recent years, dairymen in Missouri and other states have built bunker silos with concrete tilt-up sides for considerably less than this.

If this farmer decided to invest in the 20- by 50-ft. concrete stave silo and unloader, and purchased a chopper and blower, two self-unloading wagons, a mechanical feeder, and the additional concrete slab needed, he would have sufficient capital left to purchase five cows.

Should he decide to invest in the gas-tight silo, a mechanical feeder, and additional concrete slab needed, he would need to forego the purchase of a chopper and blower but could add one cow. He would then custom-hire all silage chopping and filling operations. This is not a practical alternative, because of the dependence on custom-hiring the harvesting of 800 tons of silage. If he invested in a bunker silo, he would have enough capital to purchase a chopper, blower, two second-hand dump trucks, feed bunks, and eight cows.

Net income would be increased by $2,235 if the dairyman selected the alternative of investing the $14,500 in a concrete stave tower silo, other forage equipment, and five extra cows (Table 5). The investment in a bunker silo, other forage equipment, and eight cows would add $100 less to net income. Net income would be increased by about $1,670, or $560 less, if the gas-tight silo were purchased. These calculations are based on a 30-day pasture period early in the season and feeding out of storage the rest of the year. The cost of a plastic cover was included in calculations for the bunker silo. A plastic cap costing $20 was added to expenses for the concrete stave silo. Annual depreciation, repairs, and insurance for the silos were charged at 5% of the investment for the concrete stave and gas-tight silos, and 10% for the bunker silos. A charge of 8% for depreciation and repairs was made for the investment in additional concrete slab.

If the same number of cows (from 55 to 60), and the same forage harvesting equipment, were added, the additional investment would total $14,600, $21,400, and $12,500, respectively, for the concrete stave, gas-tight, and bunker silo alternatives. The $6,600 extra investment would add $200 to net income for the gas-tight silo alternative. Increasing the number of cows to 60 rather than than to 63 for the bunker silo plan would reduce net income by $300. The bunker silo alternative would be nearly as profitable as the concrete stave silo investment, if the same percentage (85) of nutrients were preserved under both systems.

Changing the pasture system. How much would net income be increased for this 60-cow dairy farmer if he adopted a strip-grazing, green-chopping, or storage-feeding system? If he started out with silos and other equipment described for the medium level of silage-feeding situation, he could adopt any of the three systems with very little additional investment. The shift to a strip-grazing program, on farms where the pasture fields were conveniently located, would add $550 to net income (Table 6). A strip-grazing system would require 20–30 min. of labor daily.

The adoption of a green-chopping or storage-feeding system would add nearly $800 to net income when no charge is made for the extra time used in chopping forage or in feeding out of storage. The cost of all labor hired in filling the silo for storage feeding was deducted. When forage for a 60-cow herd is chopped and fed out of a self-feeding wagon, or unloaded mechanically into feed bunks, an average of 60–90 min. of man-labor is used daily. Individual dairymen will need to determine whether the time spent on this operation will cut in on other

TABLE 5

Estimated changes in receipts, expenses, and net income when $14,500 is invested in different types of silos, additional forage harvesting, and handling equipment, and more cows (200-Acre, moderately productive cropland, start with 55 cows)

Item	Size and type of silo purchased: 20 by 50 ft. concrete stave	20 by 50 ft. gas-tight	7 by 30 by 130 ft. bunker
Additional cows purchased	5	1	8
		($)	
Change in receipts			
Milk and cattle	+2,275	+ 455	+3,640
Crop sales	.....	+ 835	.....
Net change in receipts	+2,275	+1,290	+3,640
Change in expenses			
Fuel, oil, and custom work	− 174	+1,030	− 155
Labor	+ 150	.....	+ 150
Depreciation, repairs, and insurance on forage equipment	+1,229	+ 636	+1,148
Plastic cover and preservative [a]	.....	.....	+ 200
Miscellaneous dairy [b]	+ 213	+ 43	+ 354
Feed and bedding	−1,886	−2,546	− 706
Interest on additional investment	+ 508	+ 458	+ 514
Net change in expenses	+ 40	− 379	+1,505
Change in net income	+2,235	+1,669	+2,135

[a] Based on $0.50 per ton for 6-mm. polyethylene plastic cover used one season, 8 mm. green vinyl plastic cover used four seasons, or neoprene-nylon blanket used five seasons.

[b] Includes veterinary medicine, D.H.I.A., breeding, and other expenses associated with more cows.

TABLE 6
Estimated changes in receipts, expenses, and net income resulting from a change in pasture systems
(High level of silage feeding barn period, 60-cow dairy farm)

Item	Change from improved grazing to: Strip-grazing	Green-chopping	Storage-feeding
	($)		
Change in receipts [a]	.....	.....	.....
Change in expenses			
Fertilizer and seed	+107	+ 107	.....
Fuel, oil, and custom work	+ 97	+ 302	+ 204
Depreciation, repairs, and insurance on forage equipment	.....	+ 173	+ 88
Fencing	+ 30	− 60	− 60
Labor	.....	..... [b]	+ 150
Feed	−796	−1,314	−1,189
Interest on additional investment	+ 9	+ 15	+ 12
Net change in expenses	−553	− 777	− 795
Change in net income	+553	+ 777	+ 795

[a] Assume same milk production per cow for all systems.
[b] From 40 to 90 min. usually are required per day to chop and haul forage for a herd of this size.

work to such an extent that net income may be reduced. The system of storage-feeding is likely to be most economical on farms now using a loose-housing system and having mechanically unloading tower silos or self-feeding bunker silos. From 30 to 40 min. usually are required daily to feed forage when such facilities are used.

A combination of different pasture systems may be more desirable than a single system for some dairymen. It may be desirable to graze pastures early, when they are succulent and when a farmer's labor load is heavy. Storage-feeding or green-chopping are often used the balance of the pasture system. Other dairymen may find it profitable to green-chop forages early and feed out of storage after silos are filled. Dairymen need a certain amount of flexibility in their summer forage feeding practices, in order to meet year-to-year changes in climatic conditions.

SUMMARY AND CONCLUSIONS

The most profitable combination of forage methods to use depends on such factors as the size of the dairy enterprise; available buildings, equipment, and labor, and the effectiveness of various methods in preserving large quantities of high-quality feed. Dairy farmers will need to give careful consideration to the allocation of limited capital between investments in improved forage equipment, more or better cows, or other alternatives, if they are to maximize net income.

Dairymen who are equipped to store and feed only a limited quantity of silage will usually find it profitable to invest in extra silage storage needed to bring the feeding level up to 50 or more lb. per cow daily. Dairy farmers with 25 to 30 cows will not find it profitable to invest in additional silage storage needed above this medium level of silage feeding. If they harvest as many as 30 acres of first-cutting hay, they will add about $130 to net income annually if they invest $800 in a hay crimper or crusher.

Under climatic conditions similar to those in southern Michigan, dairy farmers who milk 60 or more cows often will find it profitable to harvest and store most of the first-crop alfalfa-brome grass for silage and feed only a minimum quantity (7–10 lb.) of hay per cow daily. For farms of this size, net income is increased the most when investments are made in 20- by 50-ft. concrete stave rather than gas-tight or bunker silos of equal storage capacity.

Dairymen who have no more than 25 to 30 cows and do not own chopping equipment will benefit the most by adopting a strip-grazing system. This system would require an additional investment of no more than $250 for extra fencing and would increase net income by about $360 for a 27-cow farm. Dairymen with 60-cow herds will benefit the most from using green-chopping or storage-feeding systems. Both of these systems are well-suited to farms where the forage fields are some distance from the buildings. These systems would add nearly $800 to net income when no charge is made for the extra time used in chopping green forage or in feeding out of storage. A green-chopping operation often conflicts with other work. A system of storage feeding is likely to be most profitable on farms with a loose-housing system and mechanically unloading silos or self-feeding bunker silos.

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OUR ASSOCIATION

CHANGES IN THE BY-LAWS

H. F. JUDKINS
Secretary-Treasurer, White Plains, New York

At the 53rd Annual Meeting, at North Carolina State College, Raleigh, the Board of Directors voted changes in the By-Laws. The changes have been approved by approximately 40% of the membership, while only 25% approval vote of the membership is required.

Nomination of Officers

Article II, Section 2. is now stated as follows:

"The Nominating Committee shall have five members including the immediate Past-President, one representative each from the Extension, Manufacturing, and Production sections, and one member representing commercial interests. The names of the Committee shall appear in the November issue of the Journal of Dairy Science to permit members to offer suggestions to the Committee. Such suggestions should be in the hands of the Committee not later than January 1."

Honorary Membership

Article III, Section 1(a). is now stated as follows:

"An Award of Honor may be bestowed by the the Association upon any person who has been a member of the Association for not less than 25 years and who has made a distinguished contribution to the Association."

Section 1(b). the word "Honorary" is changed to "Award of Honor."

Section 1(c). delete the words "Honorary Membership" and substitute therefor the word "Honor."

Section 1(d). delete the words "Honorary Membership" and substitute therefor "Award of Honor."

Life Membership

Article III, Section 2. is now stated as follows:

"Upon application by or on behalf of the individual concerned, the Association, through action of the Executive Board, may grant the title of Life Member to:

1. Active members who retire after having belonged to the Association for 25 years or more.
2. To any active member who has paid membership dues, to include those of a Student Affiliate, for 40 years.

Retirement is understood to mean withdrawal from full-time employment in Dairy Science or in the Dairy Industry. The status of Life Membership shall entitle the holder to all rights and privileges of regular members without the payment of annual dues."

Changes in these sections of the By-Laws became effective January 1, 1959.

BOOK REVIEW

CLINICAL ENZYMOLOGY, edited by G. J. Martin, Little, Brown and Company, Boston, 1958.

The book of 229 pages consists of the following chapters: (1) Protein Biology, G. J. Martin; (2) Chemistry of Enzymes Used Clinically, M. J. Sullivan; (3) Biochemistry of Enzymes Used Clinically, J. M. Beiler; (4) Parenteral Use of Enzymes in Medicine, H. Tanyol, W. M. Swain, and J. M. Beiler; (5) Diagnostic Use of Enzymes, G. V. Rossi; (6) Polymerases in Biology, G. J. Martin; (7) Recapitulation and Perspectives for Clinical Enzymology, G. J. Martin.

The book contains three kinds of contributions. The three chapters by Martin resemble essays, presenting his philosophy of molecular biology and the role and nature of enzymes. A second portion presents chemical and physical information on the nature of enzymes used clinically. The third portion is a relatively more detailed review of the clinical use of enzymes in therapy and in diagnosis.

For those who are interested in medical enzymology, the chapters dealing directly with this subject will make the book worth while. The authors clearly believe in the value of parenteral administration of enzymes, particularly trypsin, as treatment of choice for disorders such as acute thrombophlebitis, traumatic injury, and others. They present both favorable and unfavorable data, however, and give convincing reasons why much of the data can not be taken as meaningful. The enzymes discussed in connection with therapy are trypsin, chymotrypsin, streptokinase, ribonuclease, deoxyribonuclease, hyaluronidase, cholinesterase and, to a lesser extent, elastase and collagenase. A broader spectrum of activities is discussed in connection with enzymes as diagnostic agents. The latter chapter omits reference to errors in metabolism such as encountered in phenylketonurea, pentoseurea, and galactosemia. Although this is a new field, which is rapidly developing, its impact on clinical enzymology has already been apparent.

It is difficult to establish at which type of reader the philosophic essays of Martin are aimed. Since the subjects are not developed from their elements, it is necessary that the reader be very well informed on the phenomena under discussion. Yet the content of these chapters, while provocative and perhaps meritorious, may not be taken very seriously by those already well informed, because of the glib phrase-making style and inaccuracy and incompleteness in fact. Characteristics of Martin's style and scope of thinking is the following from page 29: "The vortex concept of the living unit. Life is a vortex in the earth stream of physiologically designed and molecular units." Obviously, this level of thinking is apart from the other chapters on clinical enzymology. For the reader who wishes to know about clinical enzymology, Martin's thoughts may be confusing. However, they represent a useful diversion for those who are so frequently concerned with the trees that the forest has not been seen.

The book also contains illustrations. The color prints of lesions before and after treatment with trypsin are to the point and effective. The photographs of Kunitzs' crystalline enzymes serve a lesser purpose, but do illustrate the beautiful simplicity and regularity of the enzyme molecule. However, it is the multiplicity and irregularity which can only be represented statistically which the editor wishes to emphasize.

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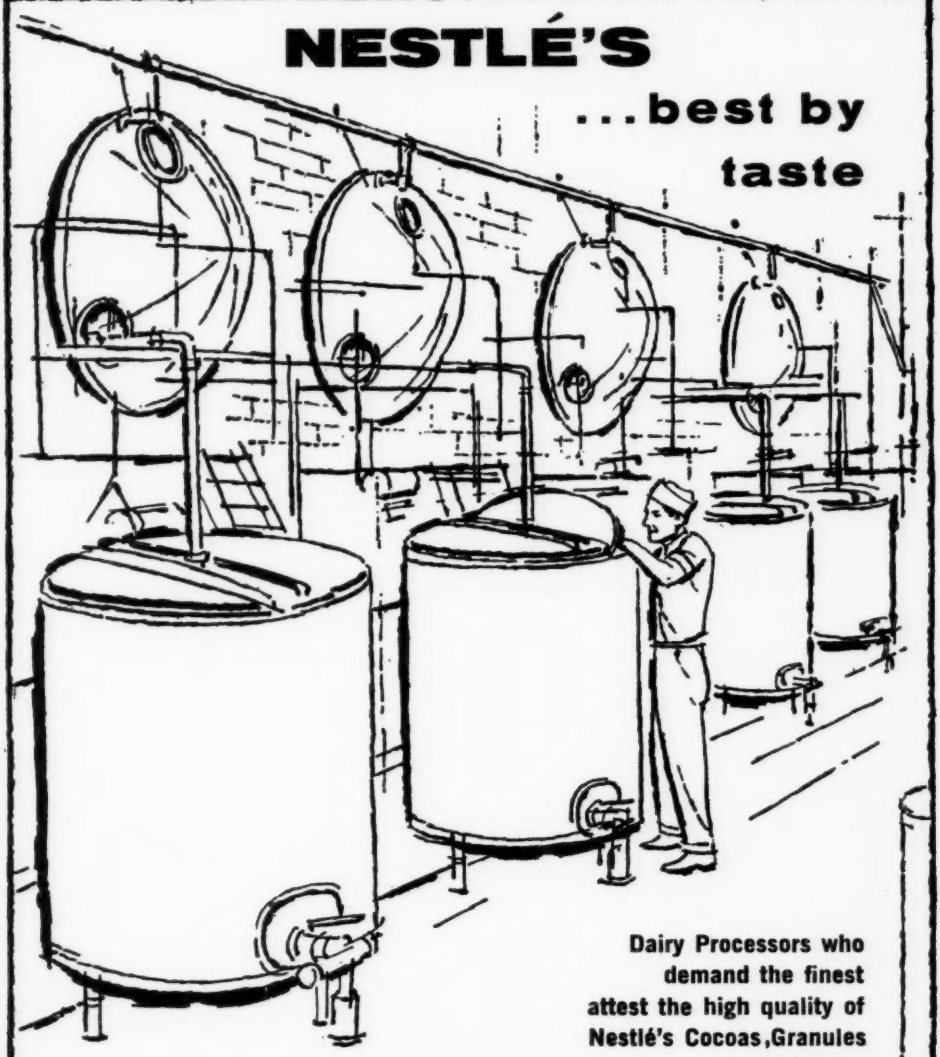

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C Protein Antiserum and Standard

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Infectious Mononucleosis Antigens and Standards

Potent

Stable

Specific

Descriptive literature available on request

Specify

DIFCO LABORATORY PRODUCTS

BIOLOGICS CULTURE MEDIA REAGENTS